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Dry matter changes and carbohydrate metabolism of germinating soybeans

Abdul Hyatt Wahab
Iowa State University

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Abdul Hyatt Wahab

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INTRODUCTION

There is a scarcity of information in the literature concerning the time sequence of specific carbohydrate changes that occur in the cotyledons, hypocotyl, radicle and apical meristem of germinating soybeans. Stored oligosaccharides are the principal substrates utilized during the early stages of soybean germination (Abrahamsen and Sudia, 1966; Pazur, Shadaksharaswamy, and Meidell, 1962). These investigators studied the metabolism of oligosaccharides in the cotyledons and embryo, but no attempt was made to study separately, the various morphological entities of the embryonic axis. Such studies on the whole seed or seedling obscure the changes occurring in the cotyledons and embryonic parts. Perhaps the first published data on the carbohydrate changes of each seed/seedling organ of the germinating soybean were those of Wahab and Burris (1971). Carbohydrates were grouped into several genera, as for example total, reducing and non-reducing sugars. Consequently, the changes in specific carbohydrates of the various seedling parts could not be ascertained.

The objectives of this study were: to investigate the time sequence of specific carbohydrate changes in the cotyledons and embryonic axis (radicle, hypocotyl, shoot apex), and to monitor the changes of fresh and dry weight of each seedling organ during germination of the soybean. In addition, the metabolic fates of several ^{14}C-sugars were studied in the seedling. Changes in carbohydrates and dry weight are discussed in relation to the role of the cotyledon in supplying metabolic components to the developing embryonic axis. It is hoped that the results obtained would serve as a standard for further basic studies on metabolism of the germinating soybean.

LITERATURE REVIEW

The physiologically mature and viable soybean seed possesses all of the chemical constituents necessary to transform it into a seedling. The seed is comprised of a number of distinct although interdependent structural entities. These entities are: a pair of cotyledons, a pair of integuments (seed coats) and an embryonic axis (hypocotyl and radicle) which constitute 90, 8, and 2% respectively of the dry weight of the seed (Encyclopedia of Chemical Technology, 1969).

Although the soybean contains mainly oil and protein, it does have an appreciable amount of structural and reserve carbohydrates. These carbohydrates vary from 20% of the dry weight of the seed in some cultivars to 34% in others (Abrahamsen and Sudia, 1966; Burrell and Wolfe, 1940; Encyclopedia of Chemical Technology, 1969; Mayer and Poljakoff-Mayber, 1966, p. 20; Pazur et al., 1962; Sato, 1921; and Street and Bailey, 1915). Investigators generally agree on the type of sugars which constitute the water soluble carbohydrate fraction of the ungerminated soybean seed. But considerable difference of opinion exists concerning the kind of sugars and polysaccharides which comprise the water insoluble residue. Some investigators have reported the presence of starch in the soybean in amounts of up to 3% of the dry weight of the seed. Others have reported their inability to demonstrate its presence (Sasaki, 1933; Sato, 1921; Street and Bailey, 1915; Swain and Dekker, 1966; Tada and Kawamura, 1963; and Von Ohlen, 1931).

Pazur et al. (1962), and Abrahamsen and Sudia (1966), investigated the changes in soluble carbohydrates during germination of the soybean. They reported that oligosaccharides comprise approximately 15% of the air-dried weight of the bean.

They agreed that sucrose [α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside], raffinose [α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside] and stachyose [O-α-D-galactopyranosyl-(1 → 6)-O-α-D-galactopyranosyl-(1 → 6)-O-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside] are the principal oligosaccharides of this group. They concluded that sucrose, raffinose and stachyose occur in a ratio of approximately 4:1:2 in the mature bean. Several workers (Abrahamsen and Sudia, 1966; Iwasa, 1937) reported the presence of small amounts of maltose, glucose, fructose, galactose and rhamnose in the ethanolic fraction of ground soybeans.

Germination

Seed germination is a means of species perpetuation. It begins with the imbibition of water and ends with the resumption of growth of the embryonic plant contained within the seed. Germination as defined by the USDA (Howell, ca. 1960) is the emergence and development from the seed embryo of those essential structures which are indicative of the ability to produce a normal plant under favorable conditions. Besides such suitable external conditions as light, moisture, temperature and aeration for germination, certain internal conditions are also required for emergence and growth. These are: available food material, metabolic systems for the mobilization, interconversion and translocation of these stored energy reserves, proper balances of growth regulators, and a structure that facilitates the transport required for life and growth. The germinating seed consists of a collection of tissues which have vastly different functions. The endosperm and cotyledons are

primarily storage tissues which senesce and die after a brief period of metabolic activity. These tissues are a source of energy from which material is mobilized and exported to the embryonic axis for growth into a seedling.

The dynamic changes accompanying imbibition and hydration have been demonstrated by McConnell (1957). He germinated radioactive wheat seeds for 5-7 days and found that 17% of the initial radiaoactivity was lost as $^{14}CO_2$ during respiration. By fractionating the seeds and seedlings before and after the germination period, he obtained increases in specific activity in some fractions and decreases in others. This indicated a redistribution of the labeled carbon, the mobility of the constituents of the seed, and their movement from the seed kernel to the stem and roots which he analyzed separately.

Carbohydrate changes

Von Ohlen (1931) using the Manchu variety of soybeans was perhaps the first investigator to conduct studies on the carbohydrate changes accompanying germination. He reported considerable quantities of non-reducing sugar in all parts of the ungerminated seed, but could not detect the presence of reducing sugar. During the first few days of germination Von Ohlen reported the appearance of reducing sugars in the embryonic axis and an increase in the level of starch in the cotyledon through the fifth day of germination. This level remained constant until the ninth day after which there was a rapid decrease. Accompanying this increase in starch level was a decrease in the amount of cotyledonary oil reserves which indicated that lipids were converted to starch.

Von Ohlen also reported that non-reducing sugars could not be detected in the hypocotyl, plumule and cotyledons after the third, fourth and seventh days respectively.

Linko, Cheng, and Milner (1960) showed with wheat germ that non-reducing sugars decrease and reducing sugars increase before the moisture level necessary for germination is reached. Toole (1924) could not detect reducing sugar in the embryo of corn until 24 hours following imbibition, when it was found in the tip of the coleorhiza and subsequently in all growing parts.

Germination is an energy requiring process dependent upon stored food material which must be mobilized and interconverted to utilizable metabolic substrates. It is generally agreed that starch, oligosaccharides and non-reducing sugars decrease rapidly from the start of imbibition to the onset of germination (Abrahamsen and Sudia, 1966; Bond and Glass, 1963; Denwyn, Whalley, and McKell, 1967; Hattori and Shiroya, 1951; Ingle, Beevers and Hageman, 1964; Linko, Cheng and Milner, 1960; McAlister and Krober, 1951; Pazur et al., 1962; Sasaki, 1933; Shiroya, 1963; Street and Bailey, 1915; Tada and Kawamura, 1963, Toole, 1924; Von Ohlen, 1931; and Wahab and Burris, 1971). Accompanying this decrease in non-reducing sugars is an increase in reducing sugars and hexoses in the growing embryo. These substrates are essential for the production of cellular energy via respiration, for amino acid metabolism and protein synthesis, cell wall synthesis, RNA and DNA metabolism.

The glyoxylate cycle and germination

It is now clear that the glyoxylate cycle by which fatty acids are converted to carbohydrates plays an important role in the energetics of germination. As early as 1859, Sachs reported on his studies of a number of oily seeds during germination. He observed that part of the oil reserves within the seed was transformed into starch. Miller (1910) stated that, during germination of the sunflower, sugars are produced from oil and are rapidly translocated to the hypocotyl where they are present in abundance. These sugars, according to Miller were the material used in the plant in the formation of new cell walls in the growing parts. Miller (1910) suggested that the oil in oily seeds breaks down into glycerine and fatty acids during germination and that carbohydrates are finally formed. Von Ohlen (1931) studying the germinating soybean, obtained data that were similar to those of Sachs and Miller. He theorized that the oil of oily seeds was converted to starch and reducing sugars during germination. Holman (1948) found that the fat content of cotyledons from Agate soybeans had decreased by 78%, 17 days after planting. During this time there was a greatly increased level of lipoxidase which catalyzes the breakdown of triglycerides to free fatty acids. Kriedemann and Beevers (1967a) studied the uptake and translocation of several sugars in the germinating castor bean. They reported a rapid increase in dry matter of the embryonic axis of the germinating seed beginning on day 4. They stated that this increase in dry matter occurred at the ultimate expense of lipids present in the endosperm of the ungerminated seed.

Until about 1960, most of the observations on lipid conversion to carbohydrates were made on oily seeds. From then on, investigators

began to study this phenomenon in cereal seeds. Dure (1960) reported that during the first 5 days of germination, growth of the axis of corn is dependent upon scutellar lipid reserves. Drennan (1962) found that the major changes occurring in grains of Avena sativa during the first day of germination were a decrease in the amounts of fat and changes in the composition of the sugars. He suggested that the metabolism of the corn embryo during the early period of germination is based on seed fats and soluble carbohydrates. Oaks and Beevers (1964) supported Dure's finding that there was a progressive loss of lipid material from cereal scutella during germination and that the stored lipid is of relevance to the carbohydrate economy of the seedling.

The biochemical pathway by which seed lipids are converted to carbohydrates is now elucidated. The reactions of this pathway and the enzymes necessary for the reactions to occur have been demonstrated in a number of oily seeds (Beevers, 1961; Bradbeer and Stumpf, 1959; Carpenter and Beevers, 1959; Kornberg and Beevers, 1957; and Yamamoto and Beevers, 1960), and also in the maize scutellum (Oaks and Beevers, 1964).

The first step in the conversion of fat to carbohydrate is the hydrolysis of triglycerides to free fatty acids and glycerol. This is made possible by a greatly increased neutral lipase activity (Beevers, 1960 and Holman, 1948). The free fatty acids are then converted to acetyl CoA by β-oxidation (Stumpf and Barber, 1957). Acetyl CoA and glyoxylate formed from the cleavage of isocitrate by isocitritase (isocitrate lyase) in the Krebs cycle combines to produce malate in the presence of malate synthetase. Malate in turn is further metabolized to sucrose.

The production of sucrose from reserve lipids occurs on a large scale

in some germinating seeds (Beevers, 1961). For example, an ungerminated castor bean contains about 260 mg of lipid (70% of its dry weight) and 15 mg of total carbohydrate. During germination at 25°C in the dark over a period of 8 days the total lipid content of the seedling falls to about 50 mg while the carbohydrate rises to 230 mg. This is in spite of the fact that sugars are being used extensively in the growth and respiration of the seedling. There is a production of more than 1 gram of sugar (mainly sucrose) for each gram of lipid consumed.

According to Kornberg and Beevers (1957), and Yamamoto and Beevers (1960), the glyoxylate cycle appears to be active in soybeans about the fifth day after the start of germination. At this time the levels of isocitritase and malate synthetase increase strikingly and are accompanied by the rapid breakdown of lipids within the cotyledons. Yamamoto and Beevers (1960) found a 7-fold increase in malate synthetase activity in the cotyledons of soybean seedlings when yeast Aceto-CoA-kinase was added to the extracts. They suggested that the capacity of crude extracts of soybean cotyledons and various plant tissues to produce malate from acetate and glyoxylate is limited by their ability to convert acetate to acetyl CoA.

Mono- and Oligosaccharide Metabolism

Because of the importance of stachyose, raffinose and sucrose in the carbohydrate metabolism of germinating seeds, this section of the literature review will be devoted to their fate. The metabolism of melibiose and maltose will also be discussed but with less emphasis. Metabolism as used here denotes such processes concerned with synthesis

and breakdown, and interconversion and translocation of these sugars during germination. The objective here is to review separately the literature on each of the more important oligosaccharides; but, due to their structural similarities and the fact that they are closely related such a goal may not be possible. Since D-fructose, D-galactose and D-glucose constitute the building blocks of these oligosaccharides, they will be included in the literature review in so far as is necessary.

Sucrose

Sucrose is the most abundant oligosaccharide in higher plants (French, 1954; Hassid, 1969; and Rorem et al., 1960) and is the most commonly found disaccharide in nature (Axelrod, 1965, p. 240). In most plants sucrose is the major organic constituent of phloem exudates (Swanson, 1959, p. 514 and Zimmermann, 1960). It is the most probable sugar that is translocated because it is the main free sugar in cotyledons of most seeds (Vitek, 1964).

Sucrose in germinating seeds Of the oligosaccharides in the ungerminated soybean, sucrose is present in the greatest amount (Abrahamsen and Sudia, 1966; Burrell and Wolfe, 1940; Encyclopedia of Chemical Technology, 1969; Iwasa, 1937; Pazur, et al., 1962; Sato, 1921; and Street and Bailey, 1915), and was still present in measurable quantities in the cotyledons of 3 day old seedlings. Abrahamsen and Sudia (1966) also reported that the sucrose content in the embryonic axis of the soybean increased during the first day of germination. They attributed this increase to the rapid metabolism of stachyose and raffinose since sucrose is a hydrolysis product of these oligosaccharides. During

germination of Avena sativa, Drennan (1962) found that sucrose was the dominant sugar throughout a 5 day germination period. Bond and Glass (1963) and Taufel, Steinbach and Hartman (1960) studied the germination of corn. They reported that with minor exceptions, the net result of carbohydrate metabolism was the synthesis of glucose and sucrose.

Sucrose is reported to be the major metabolic substrate in germinating seeds (Beevers, 1961; Bond and Glass, 1963; Kriedemann and Beevers, 1967a,b; and Taufel, Steinbach and Hartman, 1960). MacLeod and co-workers have published extensively on their investigations of carbohydrate metabolism in germinating barley (MacLeod 1951, 1952, 1960, and MacLeod, Travis and Wreay, 1953). They reported pronounced increases in sucrose and fructose after 4 days of germination and more gradual increases in glucose and maltose. These workers provided further evidence which indicates that sucrose forms the principal respiratory substrate during the early stages of growth initiation.

Translocation and transformation of sucrose Kriedemann and Beevers (1967a, b) were perhaps the first investigators to study the uptake, translocation and transformation of several sugars in castor bean seedlings. They administered uniformly labeled sucrose (sucrose-U-^{14}C), glucose-U-^{14}C and fructose-U-^{14}C to cotyledons of germinating castor bean seeds. They found that sucrose was the predominant and almost exclusive sugar in the endosperm and cotyledons respectively. Anatomical features were described which contribute to the efficiency of the cotyledons as organs of absorption and transmittal of sucrose to the embryonic axis, where hexoses were much more prevalent. The ability of the cotyledons to absorb exogenous sucrose survived removal of the endosperm from the seedling. This

sucrose absorption occurred at faster rates as the sucrose concentration was raised to 0.5 M and these rates were maintained for several hours. Removal of the embryonic axis (hypocotyl plus roots), drastically altered both the response to sucrose concentration and the time course of absorption by the cotyledons. More than 80% of the sugar normally entering the cotyledons from the endosperm was transmitted to the embryonic axis.

During sucrose uptake by the cotyledons of castor bean seedlings excised from their endosperms, small amounts of glucose and fructose accumulated in the medium. When these hexoses were supplied separately, it was found that the rate of fructose uptake exceeded that of glucose at concentrations up to 0.5M. However, sucrose uptake exceeded that of both hexoses combined, particularly at concentrations greater than 0.1M. Only minute amounts of labeled hexoses were recovered from the cotyledons after exposure to glucose-U-^{14}C or fructose-U-^{14}C; and sucrose was found to contain virtually all of the ^{14}C in the sugar fractions. In contrast to these results Nelson (1956) found that 20-30 day old soybean plants would synthesize sucrose only if supplied with fructose. Glucose-U-^{14}C when administered as a spot to a primary leaf was recovered from all parts of the stem, mainly as glucose, with some radioactivity in fructose but none in sucrose. In contrast, fructose-U-^{14}C was recovered only as sucrose evenly labeled in the glucose and fructose moieties.

The results of Kriedemann and Beevers (1967b) demonstrate that cotyledons of castor bean seedlings excised from their endosperms have the ability to synthesize sucrose from its constituent monosaccharides. Their results further demonstrate that sucrose is the preferred form of sugar accumulation in the cotyledons of germinating castor beans.

Hardy (1968) studying the fate of exogenously supplied sucrose-U-^{14}C to immature grape berries, reported an unequal labeling of glucose and fructose. Fructose became more heavily labeled than glucose indicating that an enzyme other than invertase was catalyzing the cleavage of sucrose. Hardy subsequently obtained evidence for the participation of sucrose synthesizing enzymes. He suggested that sucrose in the presence of UDP is broken down by a reversal of the sucrose synthetase reaction resulting in the production of UDP-glucose and fructose.

Sucrose synthesis in the seed During the past 30 years much has been reported on the synthesis of sucrose within the seed. The first papers to appear were merely speculative and unequivocal experimental evidence was lacking. Hassid (1969) has reviewed the subject of oligosaccharide and polysaccharide synthesis in plants. He reported that phosphorylated sugars such as D-glucose-6-phosphate and D-fructose-6-phosphate, are hydrolyzed to free sugars, causing, in some cases, the accumulation of large concentrations of D-glucose and D-fructose in plants. These phosphorylated sugars can also be converted by a series of enzymatic reactions to sugar nucleotides such as UDP-glucose which can, in turn, be interconverted by various specific epimerases and serve as donor for the formation of oligosaccharides and polysaccharides. Hassid further stated that a monosaccharide must be activated to enable the enzyme to transfer it to an acceptor for the synthesis of an oligosaccharide and subsequent formation of a polymer. From the thermodynamic point of view, nucleotide diphosphate sugars are superior donors for complex saccharide formation because they have a higher negative free energy of hydrolysis than other glycosyl

compounds.

Leloir and Cardini (1955) were the first workers to demonstrate the in vitro synthesis of sucrose by seeds. They demonstrated the presence of enzymes in wheat germ extracts which mediate the synthesis of sucrose by the following reactions:

UDP-glucose + fructose-6-phosphate → UDP + sucrose-phosphate (1)

Sucrose-phosphate → sucrose + phosphate (2)

Reaction (1) is catalyzed by sucrose phosphate synthetase (UDP glucose: D-fructose-6-phosphate 2-glucosyltransferase) and reaction (2) by phosphatase. Leloir and Cardini (1955) found that the phosphate group of the sucrose is at position 6 of the fructose moiety. They concluded that dephosphorylation with phosphatase produces a substance behaving like sucrose while mild acid hydrolysis gives free glucose and a fructose ester. This fructose ester behaves like fructose-6-phosphate when treated with acid or with isomerase plus glucose dehydrogenase. Leloir and Cardini also found that the deoxy analog of UDP-glucose was as effective as UDP-glucose, but ADP-glucose was much less effective.

Cardini et al. (1955) reported an alternative pathway by which sucrose can be synthesized. They discovered the enzyme sucrose synthetase (UDP-glucose: D-fructose 2-Glucosltransferase) from wheat germ extract which catalyzes the reaction UDP-glucose + fructose $\rightleftharpoons$ UDP + sucrose. Sucrose synthetase was also shown to be present in sugar beet, sweet sorghum and pea seeds that had been germinated for 4-5 days. The two closely related pathways by which sucrose is formed in plants have been

confirmed by several other workers (Bean and Hassid, 1955; Burma and Mortimer, 1956; and Rorem, Walker, and McCready, 1960).

Raffinose and stachyose

The addition of galactosyl moieties to a sucrose "primer" to form polymers beginning with raffinose is well known. The series begins with the first substitution occurring at the 6 position of glucose. Raffinose is second only to sucrose, as the most common sugar found in plants (Axelrod, 1965, p. 250; Bourne, Walter and Pridham, 1965; and French, 1954). Further extension of raffinose by addition of galactose to the new terminal galactose of raffinose at the 6-position gives the tetrasaccharide stachyose.

Stachyose, a classical tetrasaccharide enjoys the distinction of being the oldest and best known sugar of its class. Tanret (1913) first demonstrated the presence of stachyose in the soybean. He showed by hydrolysis that the sugar was a polysaccharide composed of 1 molecule of fructose, 1 molecule of glucose and 2 molecules of galactose. It remained to French et al. (1953) to elucidate the chemical structure and formula of the compound using the X-ray crystal density method. Soybeans are an abundant source of stachyose amounting to 3 to 4% of the dry weight of the seed (Axelrod, 1965, p. 250; French, 1954; and Pazur et al., 1962).

Stachyose and raffinose in germinating seeds During germination these oligosaccharides play an important role in the overall carbohydrate metabolism of those seeds containing them.

Stachyose and raffinose decrease rapidly and disappear altogether during germination (Abrahamsen and Sudia, 1966; Hattori and Shiroya, 1951;

Pazur et al., 1962; Sasaki, 1933; and Shiroya, 1963). Hattori and Shiroya (1951), studying germination in the Japanese black pine, reported that the concentration of raffinose decreased to zero by the time the radicle protruded the seed coat. Shiroya (1963) observed a rapid decline in the concentration of raffinose in the germinating cotton seed and an accompanying increase in the amounts of sucrose and free hexoses. In non-germinable seeds that were allowed to imbibe stachyose, raffinose and sucrose levels remained unchanged and there was no increase in the level of free hexoses.

Hattori and Shiroya (1951), Pazur et al. (1962), and Shiroya (1963) found in their studies that D-galactose was virtually absent in germinating seeds while D-glucose and D-fructose accumulated. These observations led to the conclusion that raffinose and stachyose were degraded by cleavage of the galactose moiety, and the liberated galactose was quickly utilized or interconverted to another sugar. The ways in which D-galactose could be metabolized became the subject of detailed investigations by Pazur et al. (1962) and Shiroya (1963). Pazur et al. (1962) were able to isolate and study several enzymes which participate in the metabolism of D-galactose to other compounds. These enzymes were: galactokinase which catalyze the conversion of D-galactose to α-D-galactose-1-phosphate, several uridyltransferases and UDPG-4-epimerase. Pazur et al. (1962) reported that 2 routes were available for the further metabolism of α-D-galactose-1-phosphate in the germinating bean. First the α-D-galactose-1-phosphate and UTP in the bean were converted to UDP-galactose by a UTP: galactose-1-phosphate uridyltransferase. The UDP-galactose was in turn converted to UDP-glucose by a UDP-glucose-4-epimerase and the latter

carbohydrate nucleotide was probably utilized in a variety of ways in the metabolic processes of the germinating bean. Alternatively, the α-D-galactose-1-phosphate could function as a substrate for the UDP-glucose: galactose-1-phosphate uridyltransferase which catalyzes an exchange reaction between α-D-galactose-1-phosphate and UDP-glucose to yield α-D-glucose-1-phosphate and UDP-galactose. UDP-glucose-4-epimerase was present in the bean tissue for effecting the epimerization of UDP-galactose to UDP-glucose required for continuance of the exchange reaction.

Shiroya (1963) found conspiciously high levels of α-glucosidase, α-galactosidase, β-galactosidase, β-fructofuranosidase and lower levels of α-transglucosidase, α-transgalactosidase, β-transgalactosidase, and transfructosidase in germinating cotton seeds. Levels of α-galactosidase and β-fructofuranosidase were very low in resting seeds but increased markedly in germinating seeds. Studying the fate of exogenously supplied galactose, Shiroya (1963) infiltrated this sugar into the intercellular spaces of cotyledons of germinated cotton seedlings. He detected free galactose immediately after infiltration but could not find a trace 2 hours later. When raffinose was infiltrated it decreased and completely disappeared while galactose did not appear. He concluded that there must be an active mechanism for galactose utilization in cotyledons of germinated seeds.

In contrast to the findings of Pazur et al. (1962), Shiroya (1963) reported very low levels of galactokinase and the absence of galactose phosphate in various stages of germination of cotton. Furthermore, Shiroya could not demonstrate the enzymatic epimerization of D-galactose to D-glucose mediated by UDP-glucose. Based on the absence of galactose and

melibiose [α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranose] throughout all stages of germination of the cotton seed, and the presence of much higher levels of α-galactosidase than those of β-fructofuranosidase, Shiroya concluded that raffinose and stachyose are hydrolyzed by α-galactosidase into sucrose and galactose. The latter is metabolized through the galactose utilization system present in the cotyledon of germinated seedlings.

Biosynthesis of raffinose The biosynthesis of raffinose has been studied by Bourne et al. (1965), Pridham and Hassid (1965), Rast, McInnes, and Neish (1963). These workers showed that raffinose can arise in vitro by galactosyl transfer from UDP-galactose to sucrose. An enzyme preparation from dormant broad bean (Vicia faba) seeds was incubated with a mixture of sucrose, α-D-galactose-1-phosphate (1-^{14}C) and UTP as substrates. However, if, an enzyme preparation from germinating broad bean seeds was used, synthesis of raffinose was not achieved. Hydrolysis of the trisaccharide raffinose with N HCL yielded labeled galactose while hydrolysis with invertase (β-D-fructofuranoside fructohydrolase) produced a radioactive disaccharide which was indistinguishable from melibiose on a paper chromatogram.

Recently Senser and Kandler (1967) and Tanner and Kandler (1966) tried unsuccessfully to synthesize raffinose by galactosylation of sucrose. They postulated that galactinol [O-α-D-galactopyranosyl-myo-inositol] (Brown and Serro, 1953) might be the galactosyl donor for the sugars of the raffinose family since (1) galactinol occurs only in plants which also contain these oligosaccharides, and (2) the galactosyl moiety of galactinol

showed all the kinetic characteristics of a precursor of the galactose in the raffinose sugars. In a more recent paper by Lehle, Tanner, and Kandler (1970), it was confirmed by in vivo and in vitro studies that galactinol functions as the galactosyl donor in the biosynthesis of raffinose and the other members of this family of oligosaccharide. They showed that an enzyme preparation from Vicia faba as well as from wheat germ catalyzed the formation of raffinose from galactinol and sucrose. They reported the failure of UDP-galactose to substitute for galactinol as the galactosyl donor in the reaction. Lehle et al. (1970) postulated two reaction steps for raffinose synthesis:

(1) UDP-galactose + myo-inositol $\rightleftharpoons$ galactinol + UDP

(2) galactinol + sucrose $\rightleftharpoons$ raffinose + myo-inositol

Biosynthesis of stachyose Evidence has been presented by several workers (Lehle et al., 1970; Senser and Kandler, 1967; and Tanner and Kandler, 1966) that stachyose is formed by the transfer of galactose from galactinol to raffinose in the presence of an enzyme from ripening seeds of dwarf beans. This enzyme transfers galactose specifically and with high yield. These workers found that galactinol constitutes the major galactoside in the beans during a certain maturation period and precedes stachyose in this role. They called the enzyme raffinose-6-galactosyl-transferase. The synthesis of stachyose proceeds as follows:

(1) UDP-galactose + myo-inositol $\rightarrow$ galactinol + UDP

(2) galactinol + raffinose $\rightarrow$ stachyose + myo-inositol.

Tanner and Kandler (1968) reported that the enzyme activity found per

gram of seeds is sufficient to synthesize the total amount of stachyose present in ripe seeds (15 mg/g) in less than 1 day. The galactinol content in seeds reaches a concentration of approximately 5 mM during the ripening of Vicia faba seeds. Raffinose which at the same developmental stage was present at a concentration of 0.15 mM rather seems to be the limiting factor in the biosynthesis of stachyose.

Maltose

Maltose [α-D-glucopyranasyl-(1 → 4)-α-D-glucopyranose] is found in many seeds in very small quantities. Its natural occurrence in seeds is a debatable topic since it can arise during extraction of seed for carbohydrates by autolysis of starch or by amylolysis on starch hydrolysis (MacLeod, Travis and Wreay, 1953). This disaccharide has been found in detectable amounts in wheat flour (Koch, Geddes, and Smith, 1951) and in germinating wheat (Axelrod, 1965, p. 243). MacLeod (1951) reported the presence of this sugar in ungerminated barley. Several workers reported the absence of maltose in ungerminated corn and the gradual appearance of this sugar during germination (Bond and Glass, 1963; Taufel et al., 1960). Swain and Dekker (1966) presented evidence of the existence of pathways for starch breakdown in germinating pea seedlings. They claimed that the maltose produced from starch breakdown is of a transient nature since it is readily converted to free glucose by the action of α-glucosidase.

Quillet and Bourdon (1956) are probably the only workers to have reported the occurrence of substantial quantities of maltose in the soybean. They found that at the time of flowering and pod formation, the petioles of certain varieties have up to 45% of their soluble carbohydrate fraction

in the form of maltose. Maltose is abundant also in the stems and roots.

Reynolds et al. (1958) studying the metabolism of ^{14}C-maltose, reported that, upon entering tobacco leaf disks, maltose is transformed into sucrose and starch and also provides respiratory substrates. Special features are that sucrose is always formed and that whatever the initial distribution of radioactivity between the hexose units of the substrate, the fructose and glucose combined in sucrose are equally labeled. These results suggest that the hexose units of maltose become equilibrated to form a common intermediate and are then available for further transformation. Chen and Varner (1969) reported the ready conversion of ^{14}C-maltose to sucrose in both dormant and non-dormant seeds of Avena fatua. In dormant seeds, much less sucrose was synthesized; instead, there was an accumulation of glucose.

Nigam and Giri (1960) presented evidence of the occurrence of a transglucosidase in germinating green gram (Phaesolus radiatus) which catalyzed the conversion of maltose to glucose and straight chai oligosaccharides with 1:4 glucosidic linkages such as maltotriose and maltotetraose.

Melibiose

Surprisingly little information is available on this oligosaccharide. It has been identified as a component of wheat endosperm (Koch et al., 1951), and Wykes (1952) reported its presence in small amounts in the nectar of many species. However, Wykes was unable to detect this sugar in several species of legumes. Melibiose could arise by the invertase hydrolysis of raffinose.

Fructose, glucose, and galactose

The initiation of germination and subsequent early growth of seedlings require that reserves be mobilized to provide the substrates essential for respiration, for building new cytoplasm and cell walls, and for maintaining the water potential in expanding cells. Digestion of the reserves of the storage organs to produce soluble carbohydrates and in particular hexoses is therefore an important biochemical event in seedling physiology. It is generally accepted that these hexoses in their free form constitute the major respiratory substrate and provide ultimate stores of energy for the various metabolic processes of the developing seedling.

Fructose and glucose are almost non-existent in the mature, dry, resting seed, but increase markedly during imbibition and germination. For example, Bond and Glass (1963) observed a 54-fold increase in the level of glucose during germination of corn. Fructose increased slightly but levels remained low and constant throughout the germination period. In the meristematic regions of the pea epicotyl (plumule and hook), fructose was the predominant sugar and glucose was barely detectable. In adjacent regions of internode where cell expansion occurred, glucose became the main sugar and the level of fructose declined (Maclachlan et al., 1970). These workers found that invertase and sucrose synthetase activities are directly related to the actual hexose concentrations which they observed. They reported that the distribution of invertase activity was parallel to that of glucose, while sucrose synthetase was most active in regions where fructose and sucrose were concentrated. Maclachlan et al. (1970) suggested that the principal factors controlling sugar levels in the pea epicotyl are location and relative activities of these two enzymes

utilizing translocated sucrose as substrate. In uptake studies with fructose-U-^{14}C and glucose-U-^{14}C, these workers observed that both hexoses were used equally for sucrose synthesis and interconverted to approximately the same extent.

The great demand for hexoses by the germinating seed was demonstrated by Larson and Beevers (1965) and Abdul-Baki (1969). These workers supplied glucose-U-^{14}C to cotyledons of 2 day old etolated seedlings and barley seeds that had been allowed to imbibe for a few hours respectively. Larson and Beevers found that 75% of the ^{14}C recovered following 24 hours of labeling was equally divided between alcohol insoluble residue (mainly starch), CO_2, and soluble sugars. A further 10% was recovered in homoserine, while organic acids contributed 7%. Only 5% of the ^{14}C was found as free glucose in the cotyledons but a further 17% was present in sucrose which contained 59% of its ^{14}C in the glucose moiety. In the root and shoot no ^{14}C was found in sucrose and the free glucose contained four times as much ^{14}C as fructose. Homoserine was the major soluble compound other than free sugars to become labeled; and, it accounted for 10% of the ^{14}C recovered. It appears that, as various amino acids produced from protein breakdown were being metabolized with some translocation and conversion to CO_2, glucose skeletons were simultaneously used as respiratory substrate and as a source of amino acids. Among these amino acids, homoserine predominated by virtue of its low rate of turnover.

In Abdul-Baki's studies (1969) the distribution of ^{14}C derived from glucose during the early hours of barley germination among various fractions of metabolites, indicated that 50 to 70% of the incorporated glucose appeared in CO_2. The rest of the incorporated label appeared in

hemicelluloses and starch, water-soluble ethanol-insoluble carbohydrates and a lesser extent in proteins and cellulose. These results reflect the active respiratory metabolism in the germinating seed and its importance in the production of both energy and various metabolites which are used for synthesis of cellular polymers.

Although free galactose is not found in plants, it is of widespread occurrence in combined form of a constituent of many gums, pectins, mucilages, oligo-and polysaccharides (Bonner, 1950; Roberts and Butt, 1969; and Wise and Jahn, 1952, pp. 644-646). Several workers reported the presence of D-galactose at extremely low levels in the germinating soybean and cotton seeds (Nazur et al., 1962 and Shiroya, 1963). They reported that galactose is produced from the hydrolysis of raffinose and stachyose and that it is rapidly converted to other compounds. Thimann et al. (1958) studying the metabolism of monosaccharides in oat seedlings supplied galactose-U-^{14}C to 3 day old etiolated Avena coleoptiles. They found that virtually none of the ^{14}C was converted to $^{14}CO_2$ over a three hour period. During this time 90% of the galactose taken in by the Avena coleoptiles was incorporated into 50% ethanol insoluble fraction, and the remaining 10% was found in the ethanol soluble fraction. Similar results were reported by Roberts and Butt (1969) who studied the uptake of galactose by growing maize roots. They stated that ^{14}C-1-D-galactose was rapidly taken up by excised corn root-tips and efficiently converted to hexose units in cell wall polysaccharides. The label recovered in both hydrolyzed pectin and hemicellulose was predominantly in galactose. Only α-cellulose contained appreciable amounts of labeled glucose. There was no evidence for breakdown of labeled units after incorporation into

the cell wall. Roberts and Butt reported further that, in pith cortical cells of maize, galactosyl units of hemicellulose were deposited at a maximum rate in walls approaching the end of their growth when pentose incorporation was low.

Ordin and Bonner (1957) obtained similar results as those reported by Roberts and Butt (1969) and Thimann et al. (1958). In addition, they found that galactose at a concentration of 0.01 M inhibits elongation of Avena coleoptile sections quite markedly without affecting non-auxin induced respiration. Furthermore, they found that galactose interferes with the incorporation of glucose into the cell wall polysaccharides. Studies in the metabolism of galactose indicate that this monosaccharide can be oxidized to galacturonic acid by way of its UDP derivative, and would thus contribute to cell wall formation (Altermatt and Neish, 1956). However, the concentration of D-galactose within the tissue is a critical factor for this monosaccharide to participate in elongation and growth. This was demonstrated by several workers (Burstrom, 1948; Ferguson and Street, 1957; Malca, Endo, and Long, 1967; and Roberts, Heishman, and Wicklin, 1971). They reported the growth imbibition of corn roots, roots of rye grass and wheat roots and a marked suppression of growth of tomato roots by D-galactose at concentrations as low as 0.5 mM.

Fresh Weight Changes during Germination

Germination is characterized by a rapid uptake of water which facilitates the mobilization of reserve material and the utilization of these reserves for axis growth (Ingle et al., 1964). Ingle and co-workers reported a rapid uptake of water and a consistent decrease in dry weight

of the axis of corn in the first 23 hours of germination.

In mature dry seeds, all of the tissues are shrunken, cell vacuoles are small, the nucleus is irregular, and the cell contents are plasmolyzed. Following imbibition of water, the cells become turgid, cell walls become elastic and there is an increased enzymatic activity in the seed (Toole et al., 1956). Oota (1957) showed with germinating bean seeds that water uptake consists of three distinct phases: (1) an initial steep rise largely due to diffusion, (2) a transient pause, and (3) a final steady uptake closely related to the function of the cytochrome system. Embryonic axes were among the first tissues to become hydrated during germination of lima bean (Woodstock and Pollock, 1965). Burris, Wahab, and Edje (1971) studying the effects of seed size on seedling performance in soybean, observed a doubling of the fresh weight of each seed size 24 hours after imbibition.

Opik and Simon (1963) concluded that the germination of a seed is accompanied by a rapid uptake of water and a marked rise in respiration rate. They observed a rapid initial water uptake which brings the moisture content to about 50% of the fresh weight of germinating beans 15 hours after imbibition. Maximum water uptake occurs between days 1-3 and after day three, the cotyledons begin to lose water. Wahab and Burris (1971) reported increases in respiratory activity of the cotyledons and embryonic axes of soybeans of 276 and 311% respectively 48 hours following imbibition. McAlister and Krober (1951) studying the moisture content of germinating soybeans reported that within 24 hours after planting, the moisture content of the cotyledons had risen from 13% in the dry seed to 58%. The increase in hydration was less rapid from that point on, but continued at a fairly

even rate until a moisture content of 91% had been reached 4 days after emergence. At emergence the moisture content was 80%. Hunter and Erickson (1952) reporced that a soil moisture content of about 50% was required for germination of soybean seeds. Miller (1910) studying the germination of the sunflower reported that hydration of the cotyledons was complete 10 days following imbibition.

Dry Weight Changes during Germination

The only substances normally taken up by seeds during germination are water and oxygen. In many instances substances are lost from the seed during initial stages of germination due to leaching. The initial stages of germination are consequently accompanied by a net loss of dry matter due to the oxidation of substances via respiration and leakage of soluble matter out of the seed (Mayer and Poljakoff-Mayber, 1966, p. 102). Ingle et al. (1964) reported obtaining a 23% decrease in the dry weight of the corn grain over a 5 day germination period. The dry weight of the embryonic axis decreased from 3.10 mg at the time of planting to 2.90 mg 23 hours later and increased to 8.20 mg 48 hours after imbibition. From this time on up to 5 days after planting, the embryonic axis increased in dry weight at a linear rate. The initial losses in dry weight of the embryonic axis during the first 23 hours of germination observed by Ingle et al. (1964) indicated that an initial use of endogenous substrates by the axis preceded the transfer of reserve material from the scutellum or endosperm.

Cooper and MacDonald (1970) conducted detailed studies on the energetics of early seedling growth in corn. They compared the dry matter loss of

the reserve tissue of seeds germinated in the light and dark. They reported that endosperm utilization and growth rates were similar in light and dark until the 2-leaf stage 10 days after germination. Regression of root and shoot growth on endosperm weight loss was 0.65 mg/mg and on age was 15.4 mg/day. Root and shoot growth was highly correlated ($r = 0.99$) with endosperm weight loss through 12 days.

Kriedemann and Beevers (1967a) studying the germinating castor bean, reported that dry matter was transferred through the cotyledons to the embryonic axis at a rate of 2 mg per hour after 5-6 days of germination. The loss in dry matter from the endosperm between days 4 and 7 was almost exactly matched by the increase in dry weight in the cotyledons hypocotyl and roots.

Oota, Fujii and Osawa (1953) published data on the metabolism of germinating seeds of Vigna sesquipedalis over a 6 day period. These seeds are similar to the soybeans in that they are characterized by epigeal germination. Oota *et al.* (1953) found that during germination the cotyledons show a loss of all compounds studied, while all the other embryonic organs show an increase in the various seed constituents. These workers reported a striking increase of materials in the rapidly growing hypocotyl which ceased as the hypocotyl ceased to grow. The cotyledons decreased steadily in dry weight for the first 4 days of germination. At the same time they observed a similar increase in dry weight in the other parts of the seedling and especially the hypocotyl. As the hypocotyl ceased to grow there was an accompanying rapid rise in dry weight of the epicotyl. All marked changes in the epicotyl were delayed till the onset of its growth. During the 6 day germination period

studied by Oota et al. (1953) about 20% dry weight is lost from the seeds of Vigna sesquipedalis presumably due to respiration. These investigators concluded that growth curves for each anabolic organ (plumule, epicotyl, hypocotyl and radicle) were sigmoidal, whereas cotyledons lost weight in an exponential manner. Ledig, Bortmann and Wenger (1970) in their studies on the distribution of dry matter in germinating seeds of Loblolly pine, reported that shoot and root growth were logarithmic and the ratio of their relative growth rates was a constant.

Fukui and Nikuni (1956) studied the changes in dry weight of rice seedlings during the first 10 days of germination. They observed an 84% decrease in the dry weight of the endosperm. The shoots (plumule, coleoptile, and scutellum) increased sharply in the first 8 days, then maintained a constant weight from days 8 to 10. The roots attained a maximum dry weight by day 6, then maintained a constant weight from days 8 to 10.

McAlister and Krober (1951) studied the translocation of food reserves from soybean cotyledon and their influence on the development of the plant. These workers reported a 70% total decrease in dry matter of the cotyledons of all varieties studied 28 days after emergence. There was a sharp decline in dry weight from the time of planting until 9 days after emergence in all varieties tested. After 9 days dry weight remained relatively constant. Approximately half of the decrease in dry weight occurred during the interval from planting to emergence.

Several other investigators have published on dry matter loss of cotyledons of various species during germination. Miller (1910) reported

a loss of 65% of the original dry weight of sunflower cotyledons in 13 days on plants which were grown in the light but not allowed to carry on photosynthesis. Buckner (as cited by McAlister and Krober, 1951) reported that cotyledons of garden beans lost 58%, and corn only 26% of their original dry weight when seedlings were grown to exhaustion in distilled water. Yocum (1925) working on wheat cotyledons reported a 91% loss of their original weight 25 days after planting. McAlister and Krober (1951) noted that the difference between 91% loss in dry weight for wheat cotyledons compared with 70% for soybean cotyledons, was probably due to the fact that the cotyledons of wheat seeds serve only as a source of reserve food for the seedling, while soybean cotyledons carry on photosynthesis soon after emergence.

MATERIALS AND METHODS

Soybean seeds [Glycine max (L) Merrill] of Corsoy variety were used for these studies. Seeds were produced in 1969 by The Committee for Agricultural Development at Iowa State University, Ames, Iowa. Prior to these investigations seeds were screened for uniform size using round-hole screens. Those seeds which passed through an 18/64 and held on a 16/64 screen were used. These seeds weighed 17.50 g/100 whole seeds and when tested for germination under normal conditions had a germination percentage of 98% at the end of 7 days. Throughout the experimental period, seeds at a moisture content of 6.15%, were stored in sealed glass jars at 2°C.

Fresh and Dry Weight Studies

Planting method

Four replicates of 50 seeds each were tested. Seeds were oriented with the micropyle up and planted according to the rolled paper towel method as outlined by Burris and Fehr (1971). Before planting, paper towels measuring 60 X 35 cm were immersed in running tap water until they had become saturated. Three sheets of paper superimposed on each other were used for each replicate: A flap was made lengthwise by folding down 3 cm of the top sheet. The seeds were planted along the fold 2 cm apart and the towel folded back over the seeds before the toweling was rolled.

Rolled towels were randomly placed in 3.7 liter glass jars in sets of four and the jars were covered with plastic bags to minimize evaporation. The jars were transferred to a dark growth chamber and

arranged in a completely randomized manner. Throughout the germination period the temperature inside the chamber was 28 ± 1°C. All analyses of data were done according to the procedures for a completely randomized design. The Scheffé Test (Ostle, 1963) was employed to test for significant differences between means.

Sampling

Beginning 6 hours after imbibition and continuing at 6 hour intervals through 96 hours, 50 seeds/seedlings were randomly harvested per replicate. Thereafter, seedlings were harvested at 24 hour intervals through 192 hours. Following radicle emergence, only germinated seeds were harvested. Immediately after harvesting whole seeds/seedlings were surface dried with filter paper then weighed to the nearest milligram. Following this, seeds/seedlings were dissected into cotyledons, seed coats and embryonic axes. As germination progressed and the seedling parts developed, it became feasible to separate the embryonic axis into radicle (lateral roots included), hypocotyl, and apical meristem. The apical meristem was comprised of the developing epicotyl, unifoliate leaves and shoot apex. The fresh weight of each organ was determined after which tissues were dried in a mechanical convection oven for 48 hours at 85°C. Fresh and dry weights were expressed as gram water, and gram or milligram dry matter per 50 seeds/seedlings respectively.

Carbohydrate Studies

Four replicates of 50 seeds each were planted in a manner similar to that used for the fresh and dry weight studies. Immediately after

each sampling period, the seed/seedling was dissected into its respective organs and these tissues were frozen pending carbohydrate extraction.

Carbohydrate extraction

The seed/seedling tissues were homogenized for 60 seconds in boiling 80% ethanol by means of a Sorvall Omni-Mixer adjusted to operate at full speed. The homogenate was filtered through a number 31 Whatman filter paper into a 250 ml evaporating flask. The residue was washed twice with more boiling ethanol after which it was discarded. The filtrate was concentrated under vacuum at 50°C, and the concentrate was resuspended in 5 to 10 ml of distilled water. This aqueous extract was subjected to centrifugation at 27,000 g for 30 minutes. The pellet was discarded and the supernatant was refrigerated pending total carbohydrate analysis, and qualitative and quantitative paper chromatography.

Chromatography

A modification of the multiple-ascent paper chromatography method of Robyt and French (1963) was used to effect separation of the ethanolic extract (supernatant above) into its constituent carbohydrates. Whatman No. 3 MM paper measuring 23 x 28 cm was used for all chromatographic analyses. The paper was thoroughly washed with distilled water before use to reduce the soluble carbohydrate fraction present in the filter paper.

Procedure

From 100 to 200 μl of the ethanol soluble fraction, containing not less than 200 mg total carbohydrates, was streaked along a 6.5 cm line, 2 cm from the bottom of the paper. An identical amount was streaked along a 6.5 cm line adjacent to the first line. The ends of these two lines were separated by 5 cm. A mixture of reference sugars containing equal amounts of D-fructose, D-glucose, maltose, melibiose, sucrose, raffinose and stachyose was spotted on the first line at a distance 1.25 cm from the longitudinal edge of the paper. After the samples had been applied, the paper was rolled into a cylindrical shape, stapled and placed in a chamber containing the irrigation solvent. The solvent system employed was water-n-propanol, 3:17 parts by volume. The container was made airtight by sealing the lid with high vacuum grease. Irrigation was carried out at 65°C, and sugars were satisfactorily resolved after 3 ascents of 6 hours each.

Detection of sugars

After three solvent ascents, the chromatogram was sectioned between the two lines along which the samples were streaked. This resulted in two chromatograms of equal size. One chromatogram contained both the separated reference sugars and the resolved carbohydrate fractions from the 80% ethanol soluble extract. The other one contained only separated sugars from the ethanol soluble extract. The chromatogram containing the reference sugars was sprayed to wetness using a solution of invertase containing 130 units/mg and buffered with sodium acetate at pH 4.5. The wet chromatogram was incubated in a moist chamber for 15-20 minutes at 55°C. This treatment enhances the intensity of raffinose and stachyose spots

since the invertase hydrolyzes the sucrose moiety of these oligosaccharides to glucose and fructose. The invertase treated chromatogram was dried again and developed by the silver nitrate dip method as outlined by Robyt and French (1963). This was achieved as follows: the chromatogram was immersed in a solution containing 5 ml of saturated silver nitrate per liter of acetone. The paper was dried and dipped into a solution containing 10 ml of 40% (w/v) NaOH/liter methanol, until brown spots appeared. The paper was air dried during which time the brown spots became darker and reached maximum intensity just before all of the methanol had evaporated. Following this, the paper was dipped into Kodak F-24 film fixer and immediately placed on a tray of running tap water for 1-2 hours. At the end of this washing period, sugar spots appeared as dark spots against a white background. The Kodak F-24 film fixer was prepared by dissolving 240 g sodium thiosulfate, 10 g sodium sulfite and 25 g sodium bisulfite in one liter of water. The distance that each reference sugar migrated from the line of application (origin), and the mobility of each sugar relative to D-fructose (R_{Fru} value) is presented in Table 1.

Quantitative determination of resolved sugars

The developed chromatogram was used as a guide in locating the sugars on the undeveloped chromatogram. By matching the two chromatograms, the compounds of the undeveloped one was located and this chromatogram was sectioned between the areas containing the sugars. Sugars were eluted by inserting one end of the chromatogram sections between microscope slides. The slides were taped together and placed in a shallow trough of distilled water, with the other end of

Table 1. Distance travelled by reference sugars and their mobilities relative to fructose (R_{Fru})

Reference sugar	Distance travelled from origin (cm)	R_{Fru}
D-Fructose	22.30	1
D-Glucose	19.90	0.89
Sucrose	15.40	0.68
Maltose	11.20	0.51
Melibiose	8.80	0.40
Raffinose	5.50	0.25
Stachyose	1.00	0.045

the chromatogram section suspended over a beaker. Two filter paper blanks (paper washed with water and irrigated with solvent) was included in each elution run. The trough and beakers were enclosed within a plexiglass frame so as to create a humid atmosphere. After a volume of 5-10 ml of eluent had dripped into the beaker, total carbohydrates were determined by analysis of 1 ml aliquots of the eluate with phenol and sulfuric acid as outlined by Dubois et al. (1956). Determinations were carried out in duplicate. Standard curves of each reference sugar and of a reference mixture were prepared each time the eluents were analyzed for carbohydrates. Each carbohydrate was expressed as mg sugar per 50 seeds, seedlings or seedling organs respectively.

Sugar Uptake and Translocation Studies

Radioactive sugars

Fructose-U-^{14}C(5.1 mc/ml, lot No. 522-263)

Glucose-U-^{14}C(4.0 mc/mM, lot No. 477-229)

Galactose-U-^{14}C(42.4 mc/mM, lot No. 292-064)

Sucrose-U-^{14}C(5.1 mc/mM, lot No. 580-058)

were purchased from New England Nuclear. Each radioactive sugar was added to an aqueous solution of the corresponding unlabeled carrier to give a suitable concentration and specific activity.

Sugar uptake method

Seeds were preconditioned in moistened paper towels for 18 hours before they were exposed to the labeled sugars. From 150 to 160 seeds were placed in a petri dish containing 7 to 10 ml of the radioactive sugar. Each seed was severed at the distal end (the end farthest from the micropyle) by excising 2-3 mm of the cotyledonary tissue. The seeds were arranged so that the severed end was in contact with the bottom of the petri dish. The objective in doing this was to prevent the embryonic axis from coming into direct contact with the ^{14}C-labeled sugar solution. The petri dish was enclosed in a sealed plastic box at 28 $\pm$ 1°C for four hours. The amount of ^{14}C-respired during the four hour exposure period was determined by trapping the respired CO_2 on filter papers saturated with 2N NaOH. The filter papers were placed inside the sealed plastic box and in close proximity to the seeds. This procedure was identical for each ^{14}C-sugar studied.

The specific activities of the sugars before they were exposed to the seeds were as follows:

Fructose 1.351×10^6 cpm/mM

Glucose 3.304×10^5 cpm/mM

Galactose 3.744×10^5 cpm/mM

Sucrose 3.602×10^5 cpm/mM.

Translocation study

Following the four hour incubation at 28°C, seeds were removed from the petri dish and blotted dry. Two replicates of 25 seeds each were dissected into embryonic axis, cotyledons and seed coats. The seed coats were discarded and the other tissues were frozen pending sugar extraction and chromatography. The remaining 100 seeds were planted on rolled paper towels in replicates of 2, with 25 seeds per replicate. Seeds were allowed to grow for 24 and 48 hours in the dark at 28 $\pm$ 1°C. Seedlings grown for 24 hours after the exposure were dissected into cotyledons and embryonic axes, and those grown for 48 hours following the exposure were dissected into cotyledons, hypocotyl and radicle.

Carbohydrate extraction and chromatography

Carbohydrates were extracted and chromatographed according to the procedure described earlier. Chromatograms were not developed, instead they were made into radioautograms.

Radioautograms and radioautography

Radioautograms were made by exposure of properly irrigated chromatographic paper to Kodak no-screen medical X-ray film NS-54T. Films were exposed for 30 days in the dark then developed in liquid X-ray

developer prepared in accordance with the manufacturer's instructions. Pictures of radioautographs were made using Polaroid 4 x 5 type 55 P/N black and white film and a Polaroid MP-3 camera.

Determination of radioactivity

Radioactivity of paper sections from a chromatogram was measured in a Beckman DPM 10 liquid scintillation spectrometer. The counting efficiency was about 87% as measured by including internal standards of ^{14}C-toluene in selected vials. The following liquid scintillation system was used: 10 g PPO (PPO = 2,5 diphenyloxazole) and 0.6 g POPOP (POPOP = 1,4-bis-[2-(5-phenyloxazolyl)]-benzene in two liters of toluene. Paper sections containing the radioactive compound, whose activity is to be determined, were rolled into a cylindrical shape to conform to the inner surface of a counting vial. Scintillation fluid was added to a volume of 15 ml and vials were placed in the scintillation spectrometer for counting. Radioactivity of the labeled sugar solutions and the ethanolic extracts containing the ^{14}C-sugars were determined by spotting 10 µl samples in duplicate on a filter paper. After drying the filter paper was placed in a counting vial, scintillation fluid was added and radioactivity was determined.

RESULTS

For clarity and simplicity the results of these investigations have been divided into four sections. The first and second sections pertain to changes in water content and dry matter respectively of the seed and seedling during imbibition and germination. The third section deals with the changing levels of carbohydrates, and section four pertains to the uptake and translocation of labeled carbohydrates.

Changes in Water Content during Germination

The data in Fig. 1 and Table 2 show the changes in water content of the whole seedling and various seedling organs during germination. During the first 6 hours of imbibition there was a 16-fold increase in the water content of both the cotyledons and embryonic axes. At the end of the germination period cotyledons had increased in water content from 300 mg to 14.20 g, and 56% of this increase occurred within 12 hours after imbibition. Thus, the cotyledons imbibed rapidly during the first 12 hours of germination and at a slower but steady rate from 12 through 192 hours.

The embryonic axes were sampled up to 60 hours following imbibition. During this period these tissues increased in water content from 10 mg to 11.47 g. Two distinct phases were observed to occur. The first phase of water uptake lasted from the commencement of imbibition through 18 hours. It was characterized by a rate of uptake of 15 μg of water per hour. The second phase followed the first phase and continued

Table 2. Changes in the water content of soybean seed/seedling organs and the entire seedling during germination. Results are expressed in gm water/50 seeds, seedling organs and entire seedlings, respectively

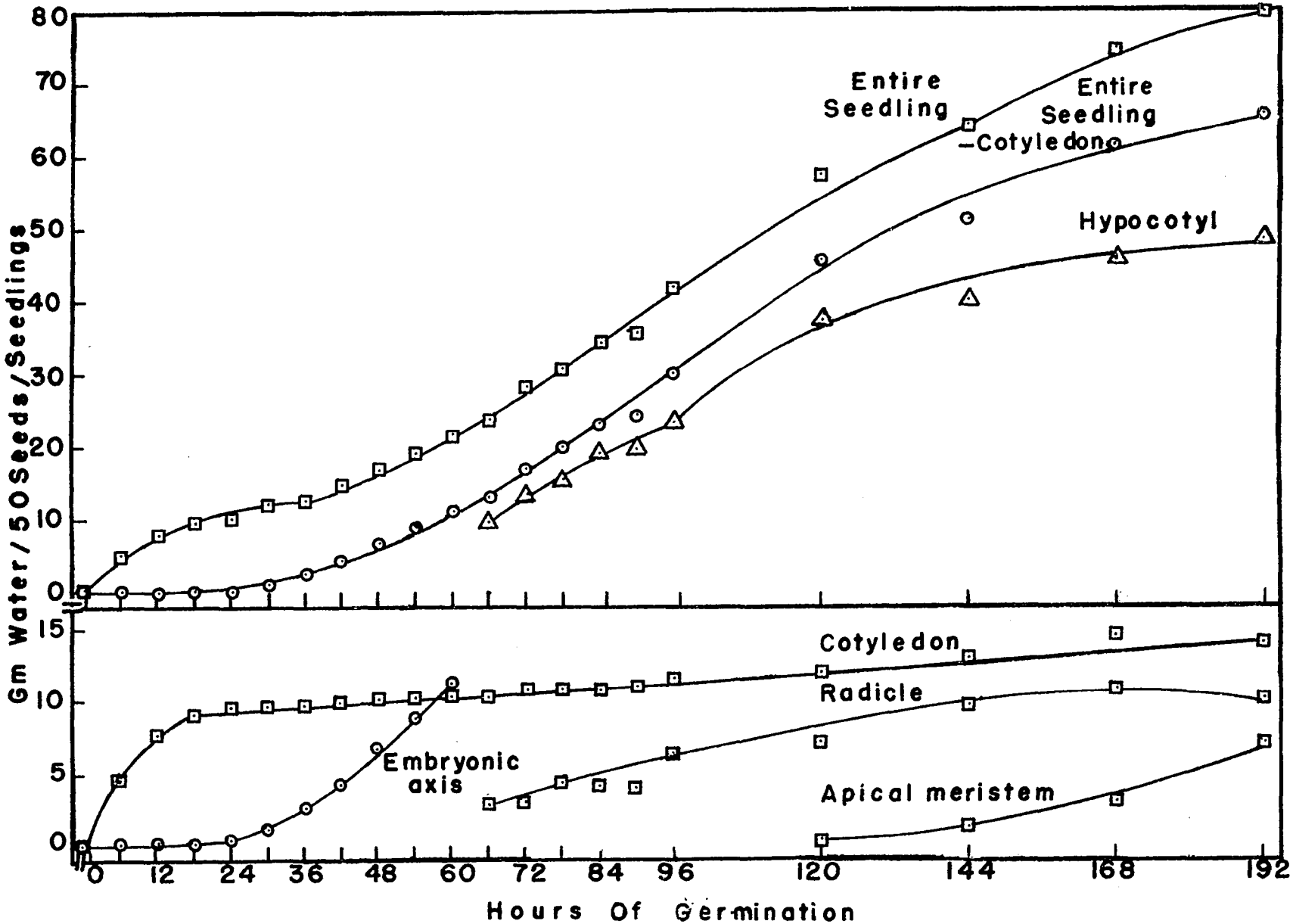
Hours of germination	Cotyledon	Embryonic axis	Hypocotyl	Radicle	Apical meristem	Entire seedling	Entire seedling - cotyledon
0	0.30	0.01	–	–	–	0.31	0.01
6	4.85	0.16	–	–	–	5.01	0.15
12	7.92	0.22	–	–	–	8.14	0.21
18	9.41	0.28	–	–	–	9.69	0.28
24	9.56	0.71	–	–	–	10.27	0.71
30	9.84	1.40	–	–	–	11.24	1.40
36	9.95	2.83	–	–	–	12.78	2.83
42	10.04	4.55	–	–	–	14.59	4.55
48	10.13	6.89	–	–	–	15.51	6.89
54	10.20	9.01	–	–	–	19.21	9.01
60	10.45	11.47	–	–	–	21.93	11.47
66	10.50	–	9.91	3.13	–	23.54	13.05
72	10.85	–	13.60	3.58	–	28.03	17.18

Table 2. (Continued)

Hours of germination	Cotyledon	Embryonic axis	Hypocotyl	Radicle	Apical meristem	Entire seedling	Entire seedling - cotyledon
78	10.74	–	15.52	4.52	–	30.79	20.05
84	11.08	–	19.02	4.42	–	34.53	23.45
90	11.29	–	19.79	4.39	–	35.45	24.17
96	11.61	–	23.29	6.49	–	41.69	30.08
120	12.17	–	37.59	7.19	0.57	57.51	45.34
144	13.07	–	40.32	9.52	1.41	64.51	51.44
168	14.50	–	46.26	10.91	3.17	76.37	61.87
192	14.20	–	48.30	10.05	7.13	79.68	65.48
$S_{0.05}$[a]	1.02	0.78	4.68	0.88	0.71	6.39	5.15

[a]Critical value at p = 0.05 which must be exceeded for means within a column to be significantly different.

Fig. 1. Changes in the water content of soybean seed/seedling organs and the entire seedling during germination.


Gm Water/50Seeds/Seedlings
80
70
60
50
40
30
20
10
0
Entire Seedling
Entire Seedling -Cotyledon
Hypocotyl
15
10
5
0
Cotyledon
Radicle
Embryonic axis
Apical meristem
0
12
24
36
48
60
72
84
96
120
144
168
192
Hours Of Germination

through the last sampling period of the embryonic axes. This phase was characterized by a rate of water uptake of 90 μg per hour.

The embryonic axis was separated into hypocotyl and radicle beginning 66 hours after seeds were planted. In addition to these organs, beginning at 120 hours it was possible to separate the embryonic axis into another entity. This entity will be referred to here as the apical meristem which is comprised of the developing epicotyl, unifoliate leaves and shoot apex.

The hypocotyl exceeded the radicle in rate of water uptake and contained the bulk of water in the entire seedling (60% of the water in the entire seedling at the end of the experiment). Water uptake data for the hypocotyl (Fig. 1) shows two distinct phases. The first phase lasted from 66 through 120 hours and was characterized by a rate of uptake of 510 μg/hr. The second phase (120 to 192 hours) was characterized by a water uptake rate of 148 μg/hr. The water content of the radicle (Fig. 1) increased at a uniform rate of 55 μg/hr from 66 through 192 hours, while there was a progressive increase in the rate of water uptake by the apical meristem (Fig. 1) from the first sampling period at 120 hours through the end of the experiment. At 144, 168, and 192 hours the water uptake rates of the apical meristem were 35, 70 and 160 μg/hr respectively.

A plot of the water levels of the entire seedling (Fig. 1) throughout the germination period reveals three distinct phases. Phase one lasted until 36 hours after imbibition and was characterized by an uptake rate of 350 μg/hr. Phase two lasted from 36 through 120 hours and was characterized by a rapid increase in water content, and an

uptake rate of 535 μg/hr. Phase three occurred from 120 through 192 hours and was characterized by the lowest uptake rate (310 μg/hr) over the entire germination period. When the water levels of the entire seedling were adjusted for the removal of the cotyledons and values plotted versus time (Fig. 1) a three phase curve was obtained. Phases two and three corresponded with those reported above for the entire seedling. However, phase one was typical of the characteristic lag phase encountered in the culturing of microorganisms. It lasted during the first 24 hours of imbibition and consisted of an uptake rate of 11 μg/hr. This demonstrates that the bulk of the water entering the seed during the first 24 hours of germination remains in the cotyledonary tissues.

Changes in the Dry Matter during Germination

The changes in dry weight of the seedling and its various organs are presented in Fig. 2 and Table 3. At the outset, the cotyledons have a greater dry weight than the embryonic axes and account for over 97% of the dry weight of the seed. Significant decreases in dry matter of the cotyledons occurred at 54, 78, 120, 144 and 192 hours after imbibition. The seedling (excluding cotyledons), increased significantly in dry matter 66, 96, 144 and 192 hours from the commencement of imbibition. At the end of the germination period studied, the embryonic axis, comprised of the radicle, hypocotyl and apical meristem accounted for 58% of the dry matter of the entire seedling (cotyledons included).

Table 3. Changes in dry matter in the soybean seed/seedling organs and the entire seedling during germination. Results are expressed in gm dry weight/50 seeds, seedling organs, and entire seedlings, respectively

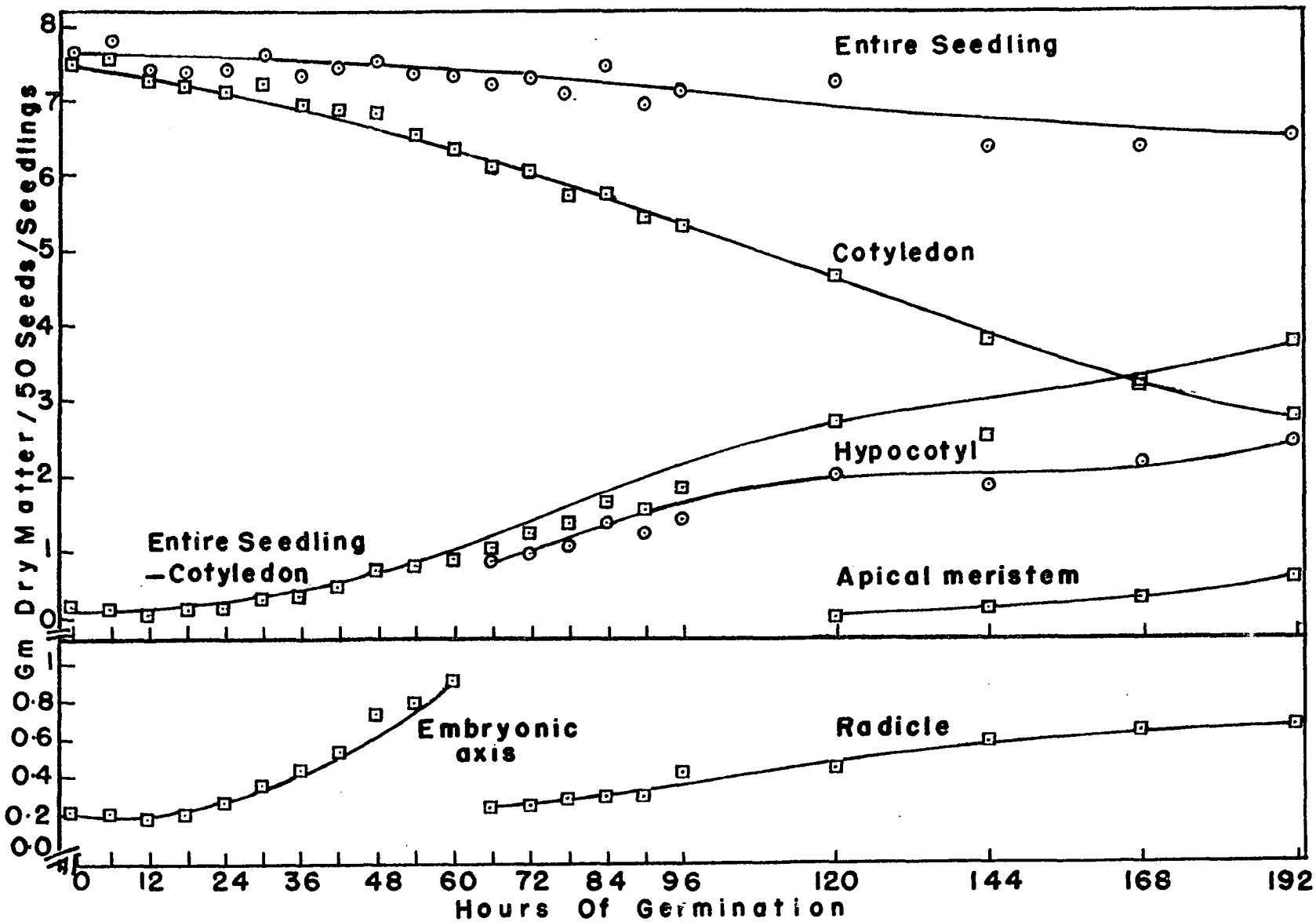
Hours of germination	Cotyledon	Embryonic axis	Hypocotyl	Radicle	Apical meristem	Entire seedling	Entire seedling - cotyledon
0	7.46	0.21	–	–	–	7.67	0.21
6	7.60	0.22	–	–	–	7.82	0.22
12	7.27	0.19	–	–	–	7.46	0.19
18	7.22	0.21	–	–	–	7.43	0.21
24	7.16	0.26	–	–	–	7.42	0.26
30	7.24	0.36	–	–	–	7.60	0.36
36	6.95	0.40	–	–	–	7.35	0.40
42	6.89	0.53	–	–	–	7.42	0.53
48	6.81	0.73	–	–	–	7.53	0.73
54	6.54	0.80	–	–	–	7.34	0.80
60	6.36	0.92	–	–	–	7.28	0.92
66	6.14	–	0.82	0.23	–	7.19	1.05
72	6.07	–	0.99	0.24	–	7.29	1.22

Table 3. (Continued)

Hours of germination	Cotyledon	Embryonic axis	Hypocotyl	Radicle	Apical meristem	Entire seedling	Entire seedling - cotyledon
78	5.75	–	1.08	0.30	–	7.12	1.38
84	5.79	–	1.38	0.31	–	7.47	1.68
90	5.41	–	1.23	0.30	–	6.95	1.55
96	5.33	–	1.43	0.43	–	7.18	1.86
120	4.66	–	2.03	0.45	0.12	7.27	2.61
144	3.84	–	1.87	0.58	0.23	6.37	2.53
168	3.21	–	2.17	0.65	0.37	6.39	3.18
192	2.72	–	2.48	0.68	0.63	6.51	3.78
$S0.05^a$	0.75	0.02	0.24	0.14	0.07	0.96	0.42

[a]Critical value at $p = 0.05$ which must be exceeded for means within a column to be significantly different.

Fig. 2. Dry matter changes in the soybean seed/seedling organs and the entire seedling during germination.


Entire Seedling
Cotyledon
Hypocotyl
Entire Seedling
—Cotyledon
Apical meristem
Embryonic
axis
Radicle
Gm Dry Matter / 50 Seeds/Seedlings
Hours Of Germination
0
12
24
36
48
60
72
84
96
120
144
168
192

Material was exported out of the cotyledons at the rate of 14.2 mg/hr during the first 36 hours of imbibition. Thereafter, rates increased sharply to 27 mg/hr and continued at this rate for the remainder of the germination period. The dry matter increase in the embryonic axis indicates a slow rate of accumulation (5.3 mg/hr) during the first 36 hours of imbibition. This was followed by a more rapid rate of dry matter increase amounting to 22 mg/hr through 60 hours. At this time the dry matter content of the embryonic axis was 4-fold greater than it was in the ungerminated seed.

The hypocotyl was first sampled 66 hours following imbibition. Two distinct phases of dry matter accumulation were observed for this organ. Phase one commenced at 66 hours and ended at 120 hours, and was characterized by a rate of dry matter increase of 22.3 mg/hr. Phase two followed phase one and continued through 192 hours. It was characterized by a markedly reduced rate of dry matter accumulation (6.25 mg/hr). This was probably due in part to the development of the apical meristem at 120 hours. This organ accumulated dry matter at a rate of 7.1 mg/hr. There were significant increases in the dry weight of the radicle at 96 and 144 hours after seeds were planted. However, no consistent trend could be observed.

Fifty dry seeds, each comprised of a pair of cotyledons and an embryonic axis weighed 7.67 g on a moisture free basis. As the seeds became hydrated, and as germination progressed there was a dry matter loss of 950 mg from the cotyledons which could not be accounted for by the dry matter content of the embryonic axis at the termination of the experiment.

Carbohydrate Metabolism

The dry seed

The ungerminated seeds contained 715.56 mg total 80% ethanol soluble carbohydrates. This constituted 8.25% of their dry weight and was distributed as follows: cotyledons 658.79 mg, embryonic axes 51.10 mg, and seed coats 5.67 mg. A chromatogram (Fig. 3) shows the various ethanol soluble sugars present in the cotyledon of the ungerminated seed. Sucrose, stachyose and raffinose were the most abundant sugars in the cotyledonary tissues and constituted 68, 24, and 5.4% respectively of the dry weight of the organ (Table 4). Fructose, glucose, and maltose were present in smaller amounts and together these sugars accounted for 2% of the total ethanol soluble carbohydrates.

The sugars in the embryonic axis of the dry seed are presented in Fig. 4. Stachyose and sucrose were the predominant sugar in these tissues and raffinose was present in smaller amounts. Trace amounts of maltose, melibiose, glucose, and fructose were also detected. The total ethanol soluble carbohydrates of the seed coats constituted 1% of their dry weight. These carbohydrates were comprised mainly of sucrose and stachyose (Fig. 5). Together, these sugars constituted 85% of the total sugars of the seed coats with the remaining carbohydrates being raffinose and fructose.

Carbohydrate changes in the seed coats following imbibition

Although it was not intended to investigate the carbohydrate changes in the seed coats during germination, the observation of ethanol

Table 4. Changes in carbohydrate levels of germinating soybean cotyledons. Results are expressed in mg carbohydrate/50 pairs of cotyledons

Hours of germination	Stachyose	Raffinose	Sucrose	Melibiose	Maltose	Glucose	Fructose	Total ethanol soluble carbohydrates
0	158.89	35.57	447.29	NDC	5.29	4.60	3.87	658.78
6	132.11	27.18	289.41	NDC	NDC	5.31	2.67	612.56
12	120.16	16.80	319.30	1.27	NDC	7.46	3.41	574.65
18	155.63	35.86	377.29	2.63	NDC	10.51	9.50	599.95
24	134.02	27.48	354.29	1.34	NDC	10.55	3.86	543.58
30	76.36	18.69	425.03	2.39	NDC	9.42	9.14	485.76
36	64.36	15.36	323.65	1.31	NDC	4.81	3.81	439.95
42	40.00	11.46	282.03	2.69	NDC	6.74	11.08	328.30
48	26.50	11.30	192.86	2.25	NDC	6.39	4.11	323.64
54	18.44	9.26	160.13	2.72	NDC	7.56	3.91	229.91
60	17.94	7.35	117.62	0.90	2.35	5.10	8.20	177.90
66	9.81	5.38	87.78	1.63	1.78	6.74	5.63	131.13
72	9.44	3.53	69.15	1.34	2.05	6.06	4.02	115.25

Table 4. (Continued)

Hours of germination	Stachyose	Raffinose	Sucrose	Melibiose	Maltose	Glucose	Fructose	Total ethanol soluble carbohydrates
78	8.19	5.40	64.59	1.94	1.69	7.92	4.65	120.93
84	9.16	5.71	64.77	2.65	NDC	9.26	6.52	106.38
90	6.87	3.31	65.37	1.91	NDC	7.66	5.07	119.39
96	4.58	NDC	58.35	0.94	NDC	9.00	6.93	107.31
120	8.17	NDC	88.18	NDC	NDC	7.21	19.83	143.26
144	NDC	NDC	47.99	NDC	NDC	10.78	10.36	97.23
168	NDC	NDC	39.20	NDC	NDC	6.91	15.90	89.45
192	NDC	NDC	28.43	NDC	NDC	6.69	15.71	75.61
$S0.05^a$	25.73	7.54	73.41	-[b]	-[b]	5.47	6.98	97.58

NDC: Not detectable on chromatogram.

[a]Critical value at p = 0.05 which must be exceeded for means within a column to be significantly different.

[b]No analysis of variance was done for these sugars.

Fig. 3. Multiple ascent chromatogram showing the sugars present in soybean cotyledons before imbibition and thereafter at 24 and 48 hours.

R is a mixture of D-fructose (FRU), D-glucose (GLU), sucrose (SUC), maltose (MALT), melibiose (MELI), raffinose (RAF), and stachyose (STACH).

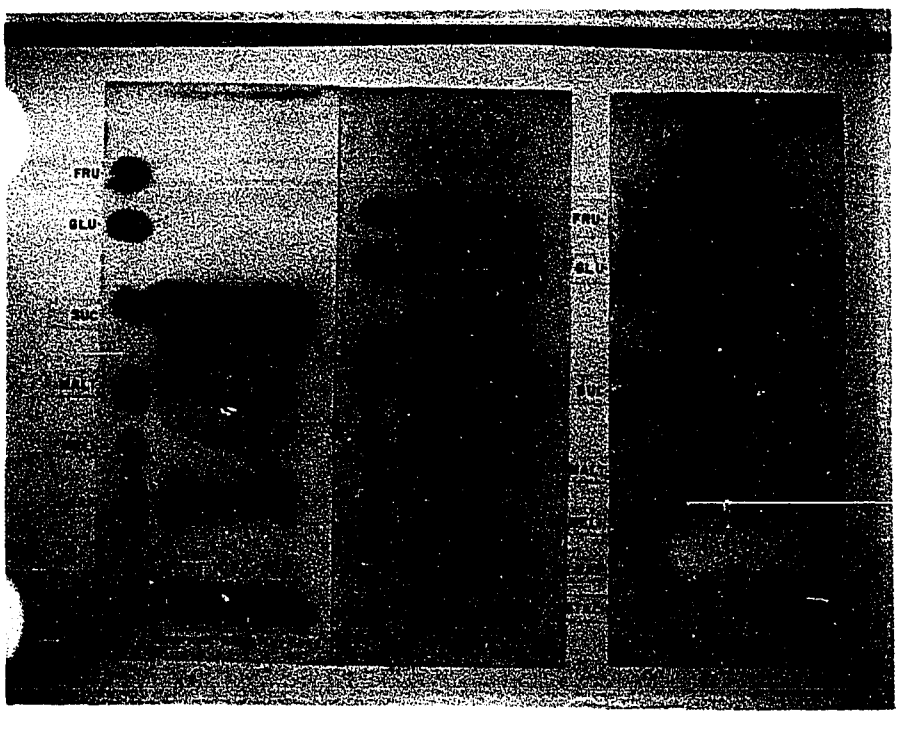


Fig. 4. Multiple ascent chromatogram of the sugars present in the embryonic axis of soybean before imbibition and thereafter at 24 and 48 hours.

R is a mixture of D-fructose (FRU), D-glucose (GLU), sucrose (SUC), maltose (MALT), melibiose (MELI), raffinose (RAF), and stachyose (STACH).

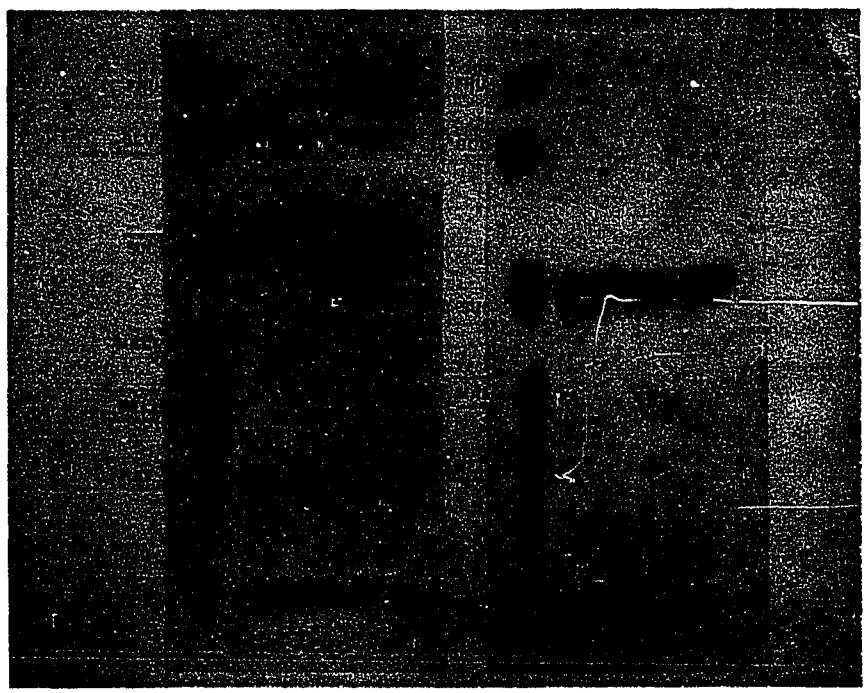


Fig. 5. Multiple ascent chromatogram of the sugars present in the seed coats of soybean before imbibition and thereafter at 24 hours.

R is a mixture of D-fructose (FRU), D-glucose (GLU), sucrose (SUC), maltose (MALT), melibiose (MELI), raffinose (RAF), and stachyose (STACH).

soluble sugars in these tissues prior to imbibition prompted further investigations. Seed coats were examined for carbohydrates at 24 hours following imbibition. The ethanol soluble sugars present in these tissues are shown in Fig. 5. Raffinose was present in trace amounts but no fructose and glucose could be detected. Total ethanol soluble carbohydrates had decreased from 4480 mg per 50 seed coats prior to imbibition to 2684 mg, 24 hours following imbibition. These sugars were comprised almost entirely of sucrose and stachyose in a 1:1 ratio.

Carbohydrate changes in the cotyledon during germination

The total carbohydrate content of the cotyledons at the initiation and termination of the experiment was 658.78 and 75.61 mg respectively. The changing levels of all the sugars are presented in Figs. 6 and 7 and Table 4. Total ethanol soluble carbohydrates of the cotyledons decreased immediately following imbibition. Levels decreased significantly at 24, 36, 42, 54, and 66 hours. At these intervals total soluble sugars had decreased by 18, 33, 50, 63, and 80% respectively. An increase in the amount of total sugars was observed between 96 and 120 hours. During this interval these sugars increased from 107 to 143 mg. Following this, total ethanol soluble carbohydrates decreased steadily through the end of the experiment. At this time these sugars had decreased by 90% of their original value prior to imbibition.

Stachyose was the second most abundant oligosaccharide in the cotyledons after sucrose. This sugar decreased significantly 12 hours following imbibition and increased to essentially its initial level 6 hours later (Fig. 6, Table 4). Stachyose levels decreased

Fig. 6. Changes in the levels of total ethanol soluble carbohydrates, sucrose, and stachyose in cotyledons of germinating soybeans.

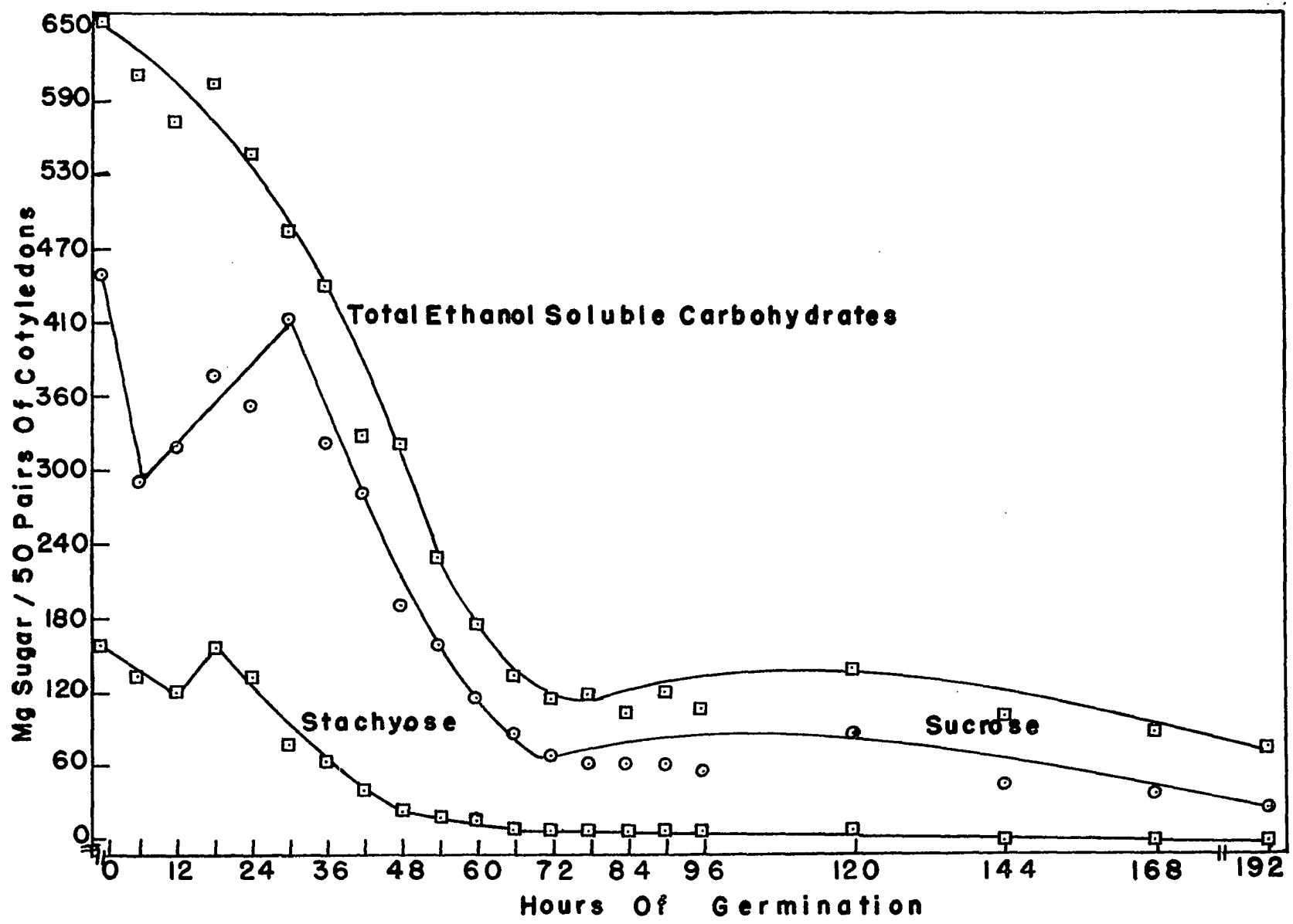
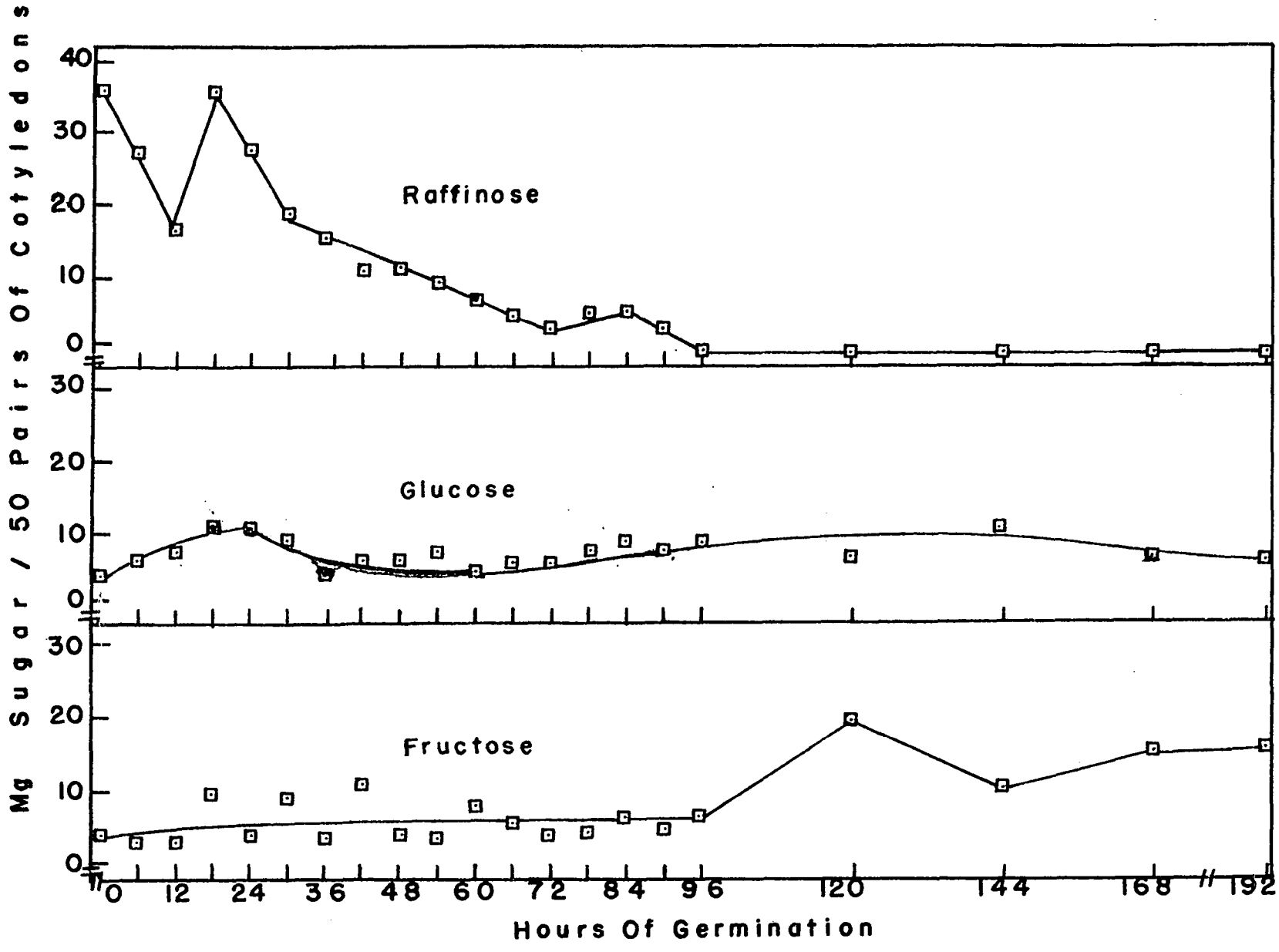

650
590
530
470
410
360
300
240
180
120
60
0
Mg Sugar / 50 Pairs Of Cotyledons
Total Ethanol Soluble Carbohydrates
Stachyose
Sucrose
0
12
24
36
48
60
72
84
96
120
144
168
192
Hours Of Germination

Fig. 7. Changes in the levels of fructose, glucose and raffinose in cotyledons of germinating soybeans


Mg Sugar / 50 Pairs Of Cotyledons
Raffinose
Glucose
Fructose
0
10
20
30
40
0
12
24
36
48
60
72
84
96
120
144
168
192
Hours Of Germination

markedly between 18 and 30 hours. At this time, 52% of the original amount of this sugar in the cotyledon was metabolized. This decrease occurred at the rate of 6.6 mg/hr. The cotyledonary tissues continued to lose stachyose rapidly until 66 hours after imbibition. At this time 94% of the stachyose present at the initiation of the experiment was utilized. Stachyose was observed to remain constant from 66 through 120 hours after which this sugar could not be detected in the cotyledons.

Although raffinose constituted only 5.5% of the total ethanol soluble carbohydrates within the cotyledon, it was the third most abundant sugar in this organ. As in stachyose this sugar decreased rapidly during the early hours of hydration but increased to its original value 18 hours following imbibition (Fig. 7, Table 4). Thereafter, a marked decline in the level of this trisaccharide occurred. Two distinctly different rates of utilization were observed. A rate of 1.2 mg/hr was recorded between 18 and 42 hours, followed by a decreased rate of 171 μg/hr over the next 48 hours. During the first 42 hours of imbibition 70% of the original raffinose was utilized and this sugar was not detected after 90 hours of imbibition.

Sucrose was the most abundant sugar in the cotyledonary tissues of soybeans and constituted 68% of the total ethanol soluble carbohydrates. This oligosaccharide decreased from its original value by 35%, 6 hours following imbibition (Fig. 6, Table 4). This was followed by an increase to essentially 100% of its original value 30 hours after seeds were allowed to imbibe. When the levels of sucrose were plotted as a function of time, a biphasic curve was obtained for the period

30 through 192 hours. Phase one occurred from 30 to 72 hours and was characterized by a rate of sucrose utilization of 8.5 mg/hr. Phase two followed phase one and was characterized by a rate of sucrose utilization of 340 μg/hr. At 60 hours following imbibition 75% of the sucrose within the cotyledons had been metabolized, and at the termination of the experiment only 6% of the original amount was observed. A characteristic increase in sucrose level similar to the increase in the level of total sugars was observed between 96 and 120 hours. Sucrose increased from 58 to 88 mg during this time.

The levels of free glucose and fructose within the cotyledonary tissues during germination are presented in Fig. 7 and Table 4. Glucose level was lowest in these tissues prior to imbibition, and increased significantly 18 hours following imbibition. At this time, glucose had increased by 228% of its original value in the cotyledon of the dry seed. Although fluctuating from 5 to 11 mg this monosaccharide remained at essentially the same level between 18 and 192 hours.

Fructose levels were lowest in the cotyledonary tissues immediately following imbibition. An erratic but definite increase followed after the seeds had imbibed for 12 hours. The most rapid increase in fructose levels occurred between 96 and 120 hours. At this time fructose amounted to 20 mg or a 5-fold increase from its initial level in the cotyledon of the dry seed. Fructose levels exceeded glucose levels from 120 hours through the end of the germination period and was at least two times greater than glucose at 120, 168 and 192 hours.

Melibiose could not be detected on the chromatogram of cotyledonary extracts until 12 hours after imbibition. At this time this disaccharide amounted to 1.27 mg in the cotyledonary tissues of 50 seeds (Table 4). Thereafter, levels of melibiose fluctuated until 96 hours of germination, after which no melibiose could be detected in the cotyledons of germinating soybeans. At no time during which this sugar was present in the cotyledon did the level exceed 3 mg.

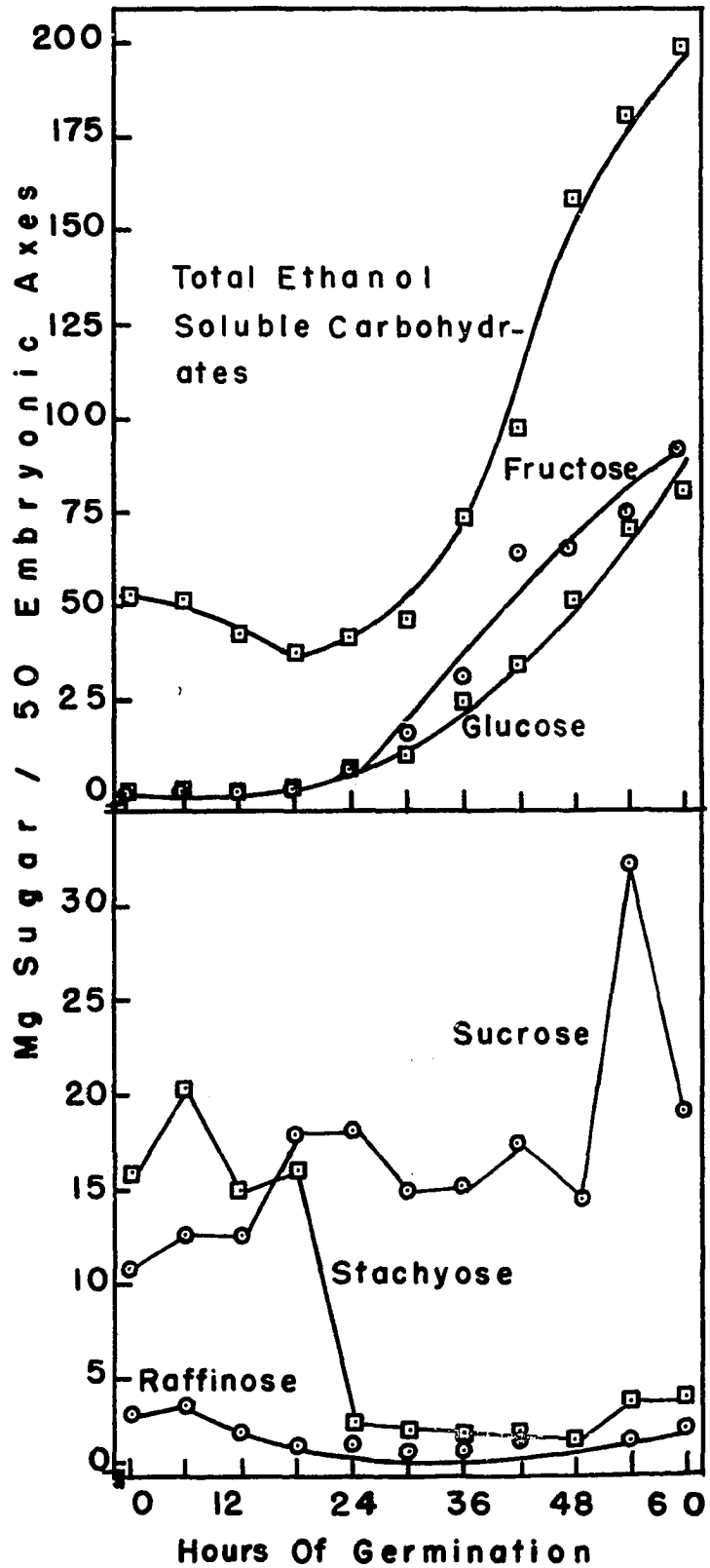
Maltose was present in the cotyledons of dry seeds in amounts larger than glucose and fructose (Table 4). However, this sugar was not detected following imbibition until at 60 hours when it amounted to 46% of the initial value before imbibition. Maltose was detected at 66, 72, and 78 hours and thereafter, could not be detected in the cotyledons of germinating seeds.

Carbohydrate changes in the embryonic axis

The data in Fig. 8 and Table 5 show the changing levels of carbohydrates within the embryonic axis during germination. Total ethanol soluble carbohydrates decreased during the first 30 hours of imbibition, and increased markedly thereafter at a rate of 5 mg/hr. At the end of the sampling period (60 hours), the embryonic axis had increased in total carbohydrate content by 400% of its initial value prior to imbibition.

Stachyose was the predominant sugar in the embryonic axis of the dry seed. Levels of this tetrasaccharide increased significantly during the first 6 hours of imbibition then decreased significantly thereafter. The lowest level of stachyose in the embryonic axis was

Fig. 8. Changes in the levels of total ethanol soluble carbohydrates, fructose, glucose, raffinose, stachyose and sucrose in the embryonic axis of germinating soybeans.


Mg Sugar / 50 Embryonic Axes
200
175
150
125
100
75
50
25
0
Total Ethanol Soluble Carbohydrates
Fructose
Glucose
30
25
20
15
10
5
0
Sucrose
Stachyose
Raffinose
0
12
24
36
48
60
Hours Of Germination

observed 48 hours after imbibition. At this time 90% of the initial amount of stachyose had been metabolized.

Raffinose constituted 6% of the total ethanol soluble carbohydrates in the embryonic axis of the ungerminated seed. This sugar increased slightly 6 hours following imbibition and decreased to essentially its original value at the end of 60 hours.

Next to stachyose, sucrose was the most abundant sugar in the embryonic axis. This situation was reversed 18 hours after imbibition at which time there was a significant increase in sucrose level from 11 to 18 mg. Sucrose levels fluctuated without any significant changes between 18 and 48 hours. A marked increase was observed from 48 to 54 hours at which time sucrose level was 3-fold greater than it was in the dry seed. Following this increase, was a significant decrease between 54 and 60 hours.

Free glucose was undetectable in the embryonic axis of the dry seed. This monosaccharide was first observed in the embryonic axis 6 hours following imbibition. Levels increased thereafter through 60 hours at which time there was 80 mg of glucose in the embryonic axis. This increase occurred in two phases (Fig. 8). Phase one was gradual and lasted from the start of imbibition to 30 hours thereafter. Phase two was rapid and continued through the end of the sampling period for this organ. Phases one and two were characterized by rates of glucose increase of 350 μg/hr and 2.3 mg/hr respectively.

Fructose was not detected in the embryonic axis of the dry seed. This hexose was definitely present 18 hours following imbibition and was the predominant sugar in the embryonic axis thereafter. As in the

case of glucose, the increase in levels of fructose was represented by two phases. Phase one lasted through 30 hours of imbibition and was characterized by a rate of increase of 550 μg/hr. Phase two followed phase one and persisted through 60 hours. It was characterized by a rate of increase of 2.3 mg/hr.

Melibiose and maltose were detected in small amounts in the embryonic axis of the dry seed (Table 5). However, maltose could not be detected following imbibition and melibiose persisted in trace amounts through 24 hours.

Carbohydrate changes in the hypocotyl

The carbohydrates in the hypocotyl of the germinating soybean are presented in Fig. 9 and Table 6. Total ethanol soluble carbohydrates increased markedly from the initial sampling at 66 hours to 84 hours. During this time the increase was linear and occurred at a rate of 7.1 mg/hr. Levels were erratic between 84 and 120 hours at which time total carbohydrate level was the same as that observed at 84 hours. An almost linear decrease in total sugars occurred from 120 to 168 hours and this was followed by a slower rate of decrease through 192 hours. Total ethanol soluble sugars decreased at the rate of 3.0 mg/hr between 120 and 168 hours and 750 μg/hr thereafter. At the end of the experimental period there was a net loss of total ethanol soluble carbohydrates from the hypocotyl which amounted to 26 mg.

Stachyose was present in the hypocotyl when this organ was first dissected from the embryonic axis at 66 hours. This tetrasaccharide

Table 5. Changes in carbohydrate levels of the embryonic axes of germinating soybeans. Results are expressed in mg carbohydrate/50 embryonic axes

Hours of germination	Stachyose	Raffinose	Sucrose	Melibiose	Maltose	Glucose	Fructose	Total ethanol soluble carbohydrates
0	15.84	2.86	10.77	0.53	1.62	NDC	NDC	51.10
6	20.13	3.34	12.49	NDC	NDC	0.19	0.35	51.76
12	14.94	2.00	12.77	0.21	NDC	0.18	NDC	42.10
18	15.87	1.21	18.08	0.40	NDC	1.34	1.28	37.16
24	2.45	1.38	18.32	0.88	NDC	5.47	5.33	41.64
30	2.12	0.88	14.89	NDC	NDC	10.59	16.47	46.39
36	1.93	1.27	15.09	NDC	NDC	24.28	29.97	73.15
42	2.01	1.56	17.23	NDC	NDC	33.07	63.71	97.03
48	1.78	1.36	14.47	NDC	NDC	50.97	64.92	157.44
54	3.85	1.70	32.28	NDC	NDC	72.00	74.91	177.15
60	3.92	2.39	19.11	NDC	NDC	79.74	86.30	197.80

Table 5. (Continued)

Hours of germination	Stachyose	Raffinose	Sucrose	Melibiose	Maltose	Glucose	Fructose	Total ethanol soluble carbohydrates
S0.05[a]	2.83	1.06	6.59	-[b]	-[b]	11.40	13.44	25.37

NDC: Not detectable on chromatogram.

[a]Critical value at p = 0.05 which must be exceeded for means within a column to be significantly different.

[b]No analysis of variance was done for these sugars.

Table 6. Changes in carbohydrate levels of the hypocotyl of germinating soybeans. Results are expressed in mg carbohydrate/50 hypocotyls

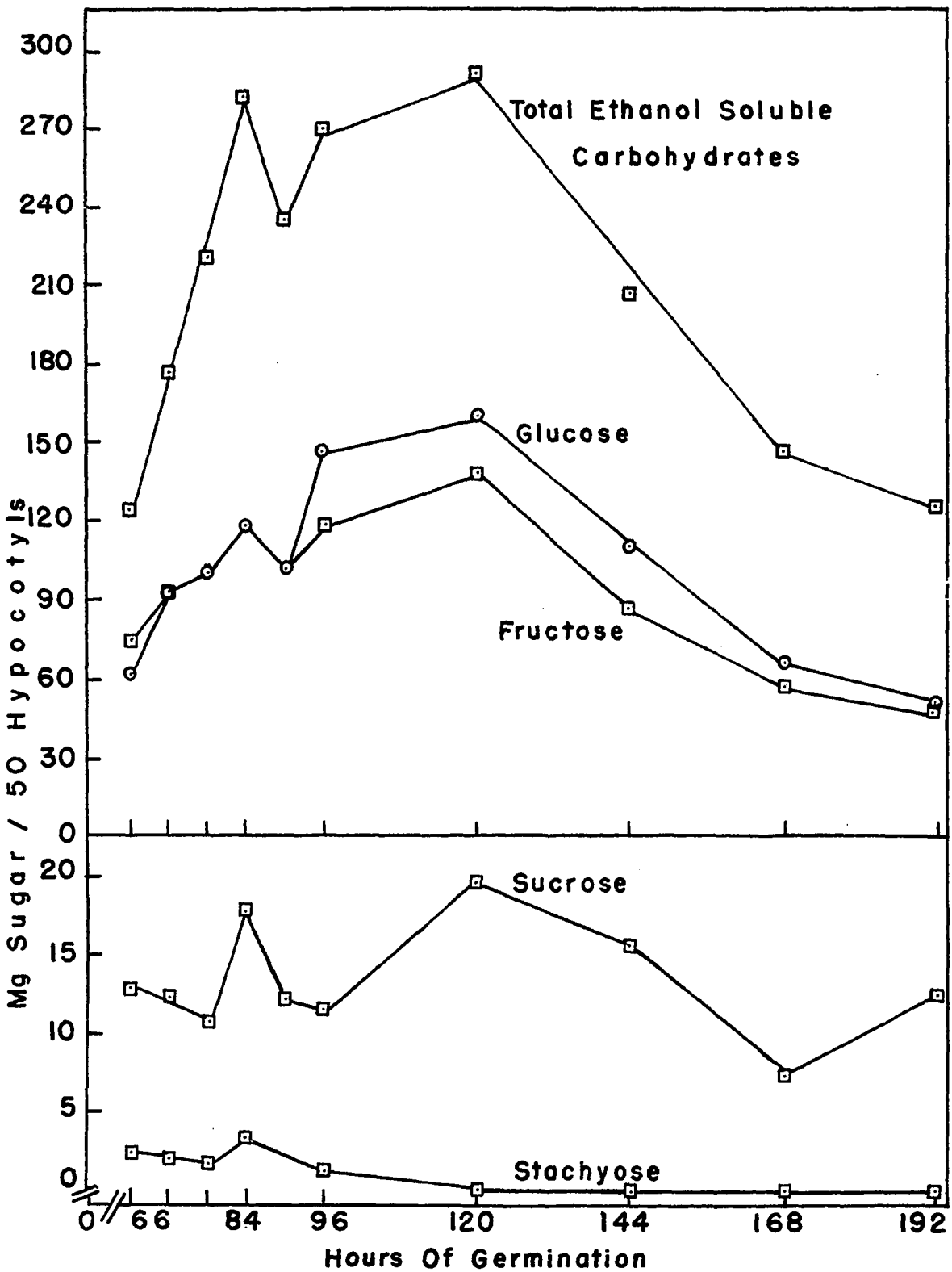
Hours of germination	Stachyose	Raffinose	Sucrose	Melibiose	Maltose	Glucose	Fructose	Total ethanol soluble carbohydrates
66	2.38	1.53	13.27	1.46	NDC	62.71	75.22	154.88
72	2.00	1.16	12.75	1.56	NDC	93.14	96.09	177.42
78	1.98	1.09	10.91	0.84	NDC	101.93	101.84	221.96
84	3.12	1.18	17.92	1.18	NDC	119.44	120.47	282.94
90	2.06	0.46	11.98	0.81	NDC	104.68	101.79	236.56
96	1.24	NDC	11.34	1.31	NDC	147.27	119.61	270.15
120	NDC	NDC	19.66	6.22	NDC	160.90	139.42	291.92
144	NDC	NDC	15.78	6.43	NDC	111.12	89.57	207.64
168	NDC	NDC	7.44	2.64	NDC	67.29	59.11	146.21
192	NDC	NDC	12.39	3.28	NDC	50.35	50.09	128.50
$S_{0.05}$[a]	1.06	0.40	4.90	-[b]	-[b]	33.38	27.06	53.18

NDC: Not detectable on chromatogram.

[a]Critical value at p = 0.05 which must be exceeded for means within a column to be significantly different.

[b]No analysis of variance was done for these sugars.

Fig. 9. Changes in the levels of total ethanol soluble carbohydrates, fructose, glucose, sucrose and stachyose in the hypocotyls of germinating soybeans.


Total Ethanol Soluble
Carbohydrates
Glucose
Fructose
Sucrose
Stachyose
Mg Sugar / 50 Hypocotyls
Hours Of Germination
300
270
240
210
180
150
120
90
60
30
0
20
15
10
5
0
0
66
84
96
120
144
168
192

persisted at low levels until 96 hours (Table 6), after which time it could not be detected. Raffinose was present in small amounts in the hypocotyl from 66 through 90 hours after which time this sugar could not be detected (Table 6).

Sucrose was the next most abundant sugar in the hypocotyl after glucose and fructose (Fig. 9, Table 6). Levels fluctuated but remained statistically unchanged from 66 to 96 hours. A sharp increase occurred from 96 to 120 hours and this was followed by a decrease through 168 hours at which time sucrose content was lowest in the hypocotyl. There was a significant increase in sucrose level between 168 and 192 hours. At this time, the amount of sucrose in the hypocotyl was essentially the same as that observed at 66 hours.

Glucose and fructose were the most abundant sugars in the hypocotyl and occurred in approximately the same amounts (Fig. 9, Table 6). Except for a decrease at 90 hours, these monosaccharides increased to a maximum value at 120 hours and decreased thereafter to similar levels at the end of the experimental period. Glucose levels were consistently higher than fructose levels from 96 through 168 hours, but the magnitude of this difference decreased as germination progressed. At the end of the experimental period these hexoses accounted for 80% of the total ethanol soluble carbohydrates within the hypocotyl.

Melibiose was detected in the hypocotyl throughout the sampling period. Levels were low and variable. The highest levels were observed at 120 and 144 hours. This coincided in point of time with the highest

levels of sucrose, glucose and fructose in the hypocotyl. Maltose could not be detected in this organ throughout the sampling period.

Carbohydrate changes in the apical meristem

The carbohydrate levels in the apical meristem are presented in Fig. 10 and Table 7. These tissues, comprised of the incompletely developed epicotyl, unifoliate leaves and shoot apex were first sampled at 120 hours. Total ethanol soluble carbohydrates increased markedly from 4 mg at 120 hours to 86 mg 72 hours later. Stachyose levels were negligible, and raffinose could not be detected after 120 hours.

Sucrose increased steadily from 120 to 168 hours (47 μg/hr) and rapidly thereafter at a rate of 500 μg/hr. Glucose and fructose were the predominant sugars in the apical meristem. Their rates of increase were approximately the same (Fig. 10), and at the termination of the experiment these hexoses accounted for 81% of the total ethanol soluble carbohydrates. Maltose was present in the apical meristem in variable but negligible amounts and melibiose could not be detected in this organ.

Carbohydrate changes in the root

The carbohydrate data for the root of the soybean seedling are recorded in Fig. 11 and Table 8. This organ was first dissected from the embryonic axis at 66 hours. Total ethanol soluble carbohydrates were variable between 66 and 90 hours and did not change significantly during this period. A sharp increase occurred between 90 and 96 hours when total carbohydrates in the root attained a maximum level. This was followed by a decrease which continued through 192 hours. At

Table 7. Changes in carbohydrate levels of the apical meristem of germinating soybeans. Results are expressed in mg carbohydrate/50 apical meristems

Hours of germination	Stachyose	Raffinose	Sucrose	Melibiose	Maltose	Glucose	Fructose	Total ethanol soluble carbohydrates
120	0.46	0.50	0.57	NDC	0.22	0.60	1.06	3.77
144	0.34	NDC	1.90	NDC	0.23	2.02	2.32	9.78
168	NDC	NDC	4.34	NDC	0.07	13.34	11.05	32.15
192	0.94	NDC	16.52	NDC	0.30	34.09	35.57	85.97
S0.05[a]	-[b]	-[b]	0.99	-[b]	-[b]	2.19	2.09	6.42

NDC: Not detectable on chromatogram.

[a]Critical value at p = 0.05 which must be exceeded for means within a column to be significantly different.

[b]No analysis of variance was done for these sugars.

Table 8. Changes in carbohydrate levels of the radicle of germinating soybeans. Results are expressed in mg carbohydrate/50 radicles

Hours of germination	Stachyose	Raffinose	Sucrose	Melibiose	Maltose	Glucose	Fructose	Total ethanol soluble carbohydrates
66	0.67	0.37	1.57	0.10	0.10	9.54	16.25	30.79
72	0.78	0.42	1.61	0.13	NDC	8.89	16.40	29.50
78	0.84	0.59	0.47	NDC	NDC	10.98	16.88	32.78
84	2.46	0.49	1.04	NDC	NDC	10.57	18.15	38.39
90	0.65	NDC	0.93	NDC	NDC	11.33	16.35	34.39
96	0.75	NDC	1.27	NDC	NDC	18.33	20.55	43.47
120	NDC	1.20	4.92	NDC	1.02	10.02	25.37	39.14
144	NDC	NDC	3.95	NDC	NDC	8.81	25.01	39.42
168	NDC	NDC	3.57	NDC	NDC	6.06	13.75	33.93
192	NDC	NDC	4.12	NDC	NDC	3.41	12.77	28.12
$S0.05^a$	-[b]	-[b]	1.49	-[b]	-[b]	9.99	5.27	9.12

NDC: Not detectable on chromatogram.

[a]Critical value at p = 0.05 which must be exceeded for means within a column to be significantly different.

[b]No analysis of variance was done for these sugars.

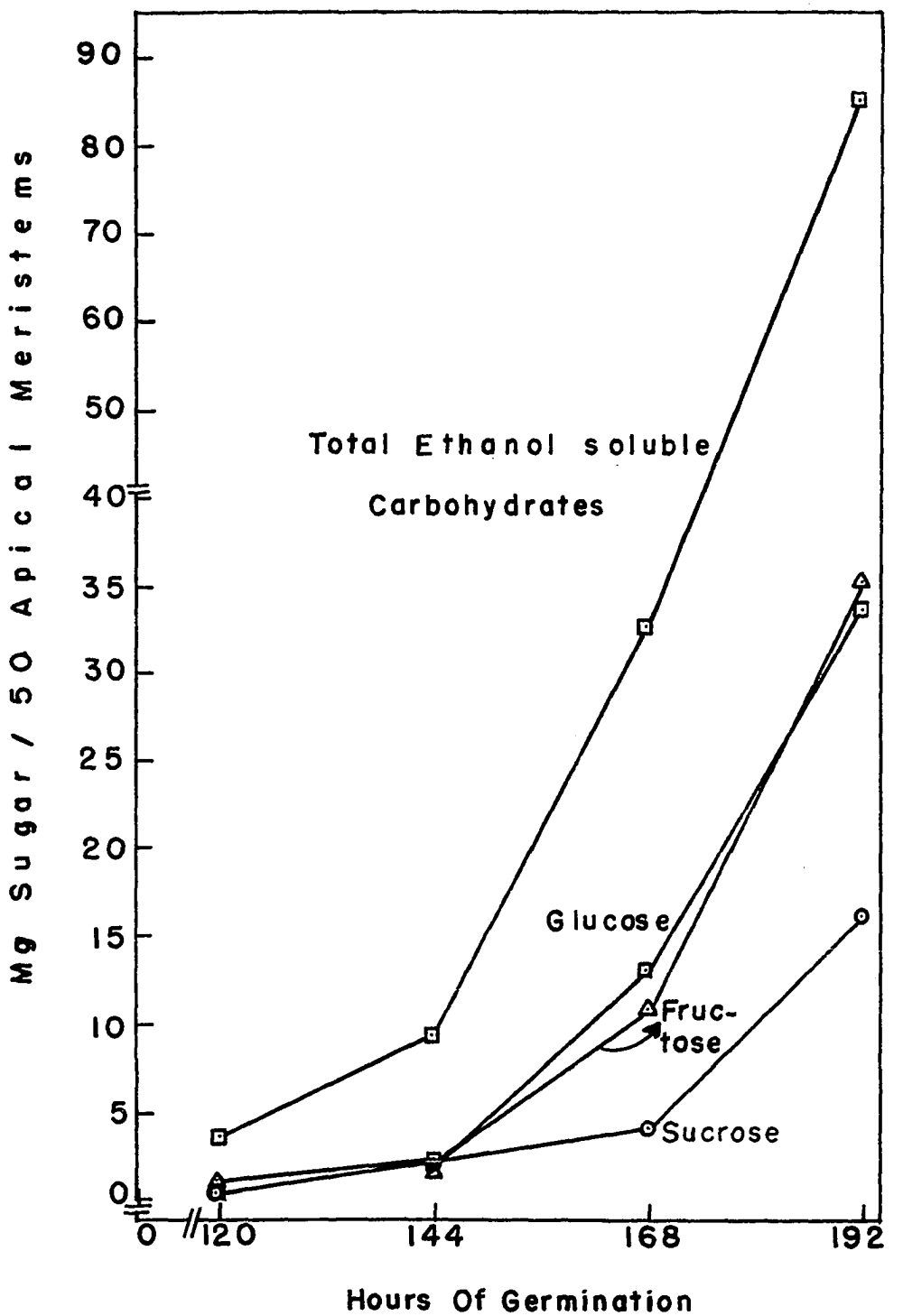


Fig. 10. Changes in the levels of total ethanol soluble carbohydrates, fructose, glucose and sucrose in the apical meristems of germinating soybeans.

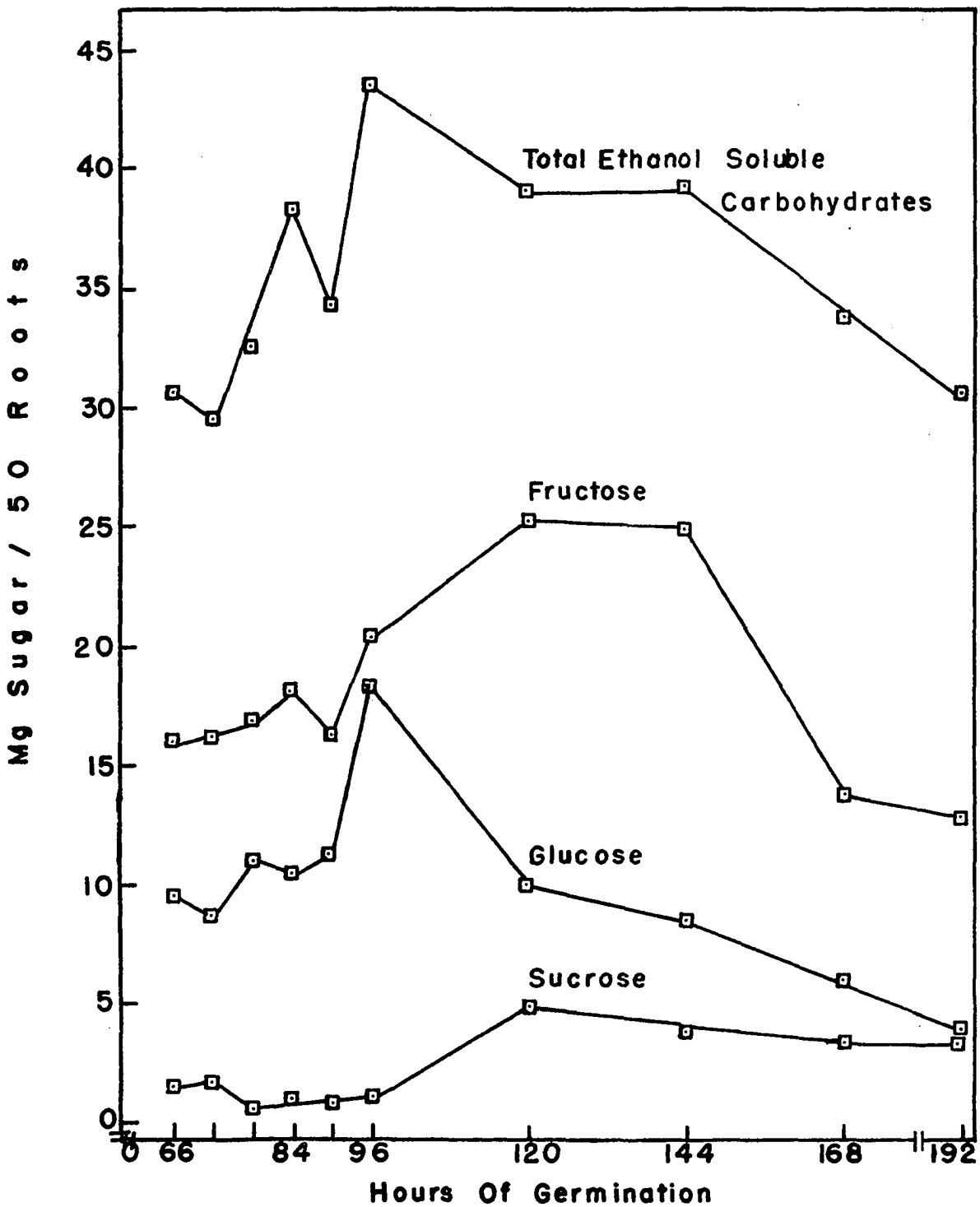


Fig. 11. Changes in the levels of total ethanol soluble carbohydrates, fructose, glucose, and sucrose in the roots of germinating soybeans.

this time the total carbohydrate level in the root was essentially the same as that observed at 66 hours.

Stachyose was detected in the root from 66 through 96 hours but not thereafter. Levels were low during most of this interval and the unusually high value reported at 84 hours might have been due to experimental error. Raffinose was detected from 66 through 84 hours and again at 120 hours. As in the case of stachyose, raffinose was present in the root of germinating soybeans in small quantities.

Sucrose was present in the root throughout the sampling period. Levels were lowest and fluctuated with no significant change between 66 and 96 hours. A significant increase occurred between 96 and 120 hours when sucrose level was highest in the root. There was no significant change in the sucrose level thereafter, and at the end of the experiment sucrose content of the root was 160% higher than when this organ was first sampled at 66 hours.

Glucose and fructose were the most abundant carbohydrates in the root, with fructose levels consistently greater than glucose levels. Glucose levels increased gradually between 66 and 90 hours, and rapidly during the following 6 hours. The level of glucose in the root reached a maximum at 96 hours. At this time this sugar had increased from its initial level by 2-fold. Glucose levels decreased significantly after 96 hours and the lowest level was recorded at the termination of the experiment. Fructose levels were twice as much as the glucose levels in the root for most of the sampling periods. When this organ was first sampled at 66 hours, fructose constituted 53% of the total ethanol soluble carbohydrates. Levels fluctuated between 66 and 78 hours

then increased to a maximum at 120 hours. This was followed by a significant decrease at 168 hours; and, at the termination of the experiment fructose level was not significantly different from the initial level observed at 66 hours.

Melibiose was detected in trace amounts at 66 and 72 hours but was not detected thereafter. Maltose too was detected in trace amount at 66 hours and was not detected again until at 120 hours. Thereafter, this disaccharide was not present in the root of germinating soybean.

Carbohydrate changes in the whole seedling

The carbohydrate data for the whole seedling (Fig. 12, Table 9). represent the sum total of the determinations for each seed/seedling organ throughout the experimental period. Total carbohydrate levels changed in the entire seedling in three distinct phases (Fig. 12). Phase one lasted from the start of imbibition to 66 hours. This phase was characterized by a decrease in total carbohydrates at the rate of 6 mg/hr, and as it terminated 56% of the total carbohydrates in the ungerminated seed had been utilized. Phase two was characterized by an increase in the level of total ethanol soluble carbohydrates from 317 mg at 66 hours to 480 mg at 120 hours. Phase three followed phase two and lasted through the end of the experiment. This phase was characterized by a decrease in the level of total carbohydrates from 480 mg to 318 mg. Thus, the 163 mg increase in total sugars occurring between 66 and 120 hours was utilized between 120 and 192 hours.

Table 9. Changes in carbohydrate levels of the whole seed/seedling during germination of the soybean. Results are expressed in mg carbohydrate/50 whole seeds/seedlings

Hours of germination	Stachyose	Raffinose	Sucrose	Melibiose	Maltose	Glucose	Fructose	Total ethanol soluble carbohydrates
0	174.23	38.26	458.05	0.53	6.91	4.60	3.87	709.89
6	152.25	30.50	301.93	NDC	NDC	5.46	2.99	664.32
12	135.10	18.80	332.07	1.48	NDC	7.55	3.41	616.75
18	171.50	35.64	395.42	3.03	NDC	11.86	10.53	637.11
24	136.48	28.85	372.61	2.22	NDC	16.03	9.19	585.22
30	78.48	19.56	439.92	2.39	NDC	20.00	25.51	532.15
36	66.29	16.63	338.74	1.31	NDC	29.09	33.78	513.10
42	42.01	13.01	299.26	2.69	NDC	39.81	75.50	425.33
48	28.27	12.66	207.33	2.25	NDC	57.36	69.03	481.07
54	22.29	10.80	186.94	2.72	NDC	79.56	78.81	407.06
60	21.85	9.74	136.73	0.90	2.35	84.83	94.49	375.69
66	12.86	7.27	102.63	3.10	1.88	78.99	97.09	316.80
72	12.23	5.14	83.51	3.03	2.03	107.92	116.51	322.17

Table 9. (Continued)

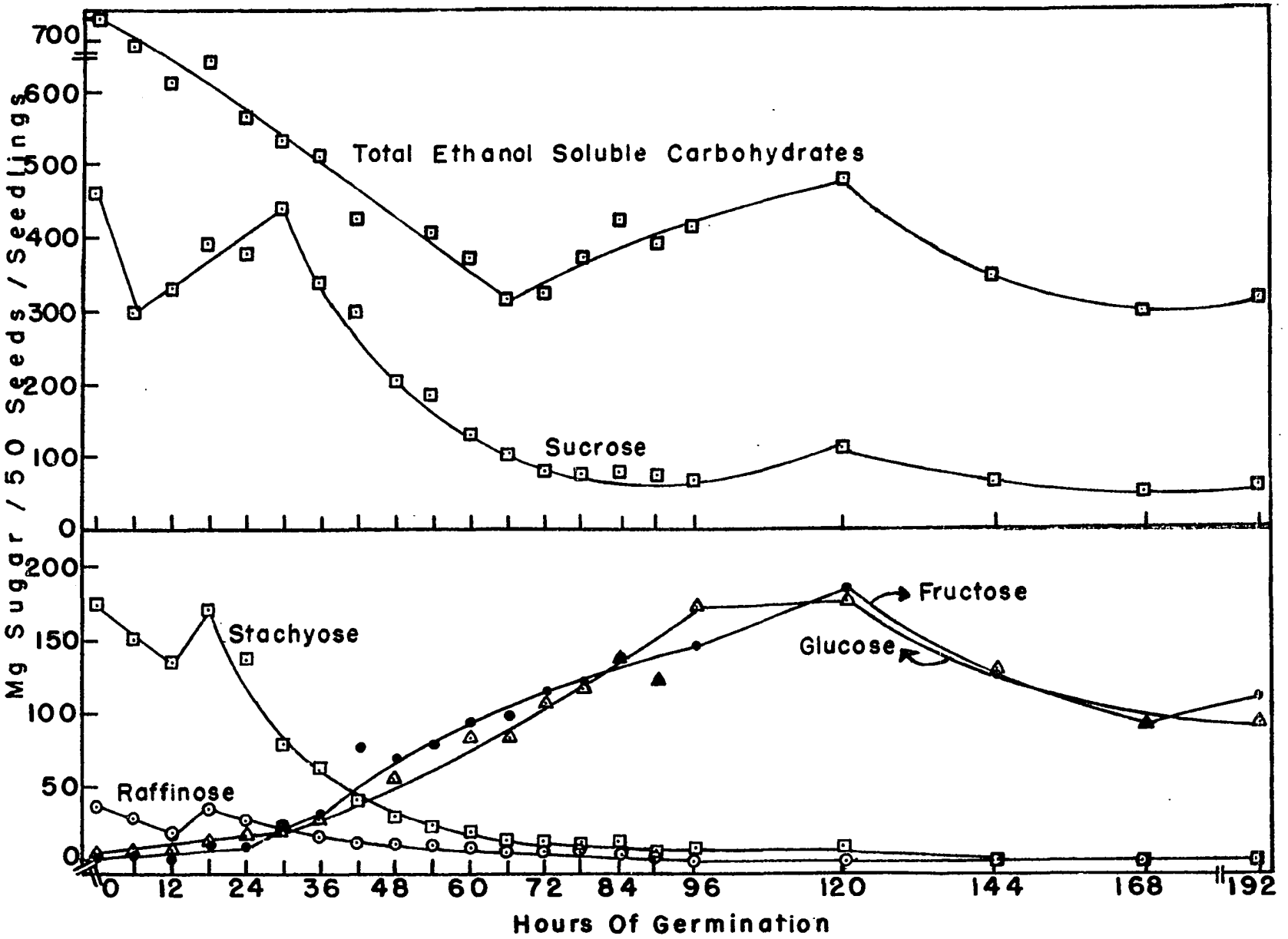
Hours of germination	Stachyose	Raffinose	Sucrose	Melibiose	Maltose	Glucose	Fructose	Total ethanol soluble carbohydrates
78	11.01	7.08	75.97	2.78	1.69	120.83	123.37	375.66
84	14.74	7.38	83.72	3.83	NDC	139.27	139.31	424.94
90	9.58	3.77	78.28	2.72	NDC	123.67	123.20	390.35
96	6.56	NDC	70.96	2.25	NDC	174.60	147.09	420.94
120	8.63	0.50	113.32	6.22	NDC	178.73	187.01	478.10
144	NDC	NDC	69.63	6.43	0.23	132.72	127.26	354.06
168	NDC	NDC	55.34	2.64	0.07	93.68	99.80	301.75
192	NDC	NDC	61.28	3.28	0.30	94.53	114.66	318.21
$S_{0.05}$[a]	25.63	7.47	74.74	-[b]	-[b]	56.35	32.85	115.28

NDC: Not detectable on chromatogram.

[a]Critical value at p = 0.05 which must be exceeded for means within a column to be significantly different.

[b]No analysis of variance was done for these sugars.

Fig. 12. Changes in the levels of total ethanol soluble carbohydrates, fructose, glucose, raffinose, stachyose, and sucrose in the whole soybean seedling during germination.


Total Ethanol Soluble Carbohydrates
Sucrose
Stachyose
Raffinose
Fructose
Glucose
Mg Sugar / 50 Seeds / Seedlings
Hours Of Germination
0
12
24
36
48
60
72
84
96
120
144
168
192
100
200
300
400
500
600
700
50
150

Stachyose levels decreased significantly 12 hours following imbibition and increased to the initial value in the dry seed 6 hours later. There was a marked decrease in the level of this sugar between 12 and 48 hours at which time 84% of the initial amount of stachyose measured in the dry seed had been metabolized. Stachyose levels continued to decline after 48 hours of imbibition and starting at 144 hours this tetrasaccharide could no longer be detected in the seedling. Raffinose decreased during the first twelve hours of germination by 51% and levels increased to essentially the original value observed in the dry seed 18 hours following imbibition. Thereafter, raffinose declined gradually and could not be detected in the seedling after 120 hours of germination.

Sucrose was the most abundant sugar in the seedling through 66 hours of germination. This disaccharide decreased markedly 6 hours following imbibition. At this time 34% of the original sucrose content of the ungerminated seed had been metabolized. Sucrose levels increased between 6 and 30 hours at which time the entire seedling accounted for 96% of the original amount of sucrose in the dry seed. Sucrose was utilized steadily at the rate of 6 mg/hr between 30 and 90 hours at which time 83% of the initial sucrose level in the dry seed was metabolized. A slight increase in sucrose levels was observed between 90 and 120 hours followed by a decrease through the end of the experimental period. At this time the seedling still contained 13% of the initial amount of sucrose observed in the ungerminated seed.

Glucose and fructose levels changed similarly during most of the germination period. Excepting for melibiose and maltose, these sugars were present in the smallest amounts during the early hours of germination. This situation was reversed starting at 72 hours when these hexoses were the predominant sugars in the seedling. Glucose levels increased gradually from 4.60 mg in the dry seed to 20 mg in the entire seedling 30 hours following imbibition. This was followed by a steady increase through 120 hours, at which time glucose amounted to 179 mg in the entire seedling thereby attaining a maximum level. Thereafter, glucose levels decreased to 95 mg at the conclusion of the experiment. Fructose, like glucose, increased gradually during the first 30 hours of imbibition. Subsequent to this, a rapid increase in the level of fructose occurred from 26 mg at 30 hours to 187 mg at 120 hours. This hexose decreased thereafter and amounted to 115 mg at the end of the experiment.

Melibiose was detected in the dry seed in small amounts and was not observed 6 hours following imbibition. This disaccharide was again detected at 12 hours and was present in the seedling through 192 hours, although in small amounts. Maltose was present in the dry seed in amounts larger than either glucose or fructose. It could not be detected during the 54 hour period following imbibition but was detected again from 60 through 78 hours. Maltose was absent in the seedling from 84 through 96 hours, and thereafter, was present in small amounts.

Uptake and Translocation of ^{14}C-Labeled Sugars

^{14}C-Activity of filter papers

The activities of ^{14}C derived from $^{14}CO_2$ and trapped on filter papers moistened with NaOH during the exposure period are presented in Table 10. These results demonstrate that each labeled sugar was incorporated by the seedling tissues and served as respiratory substrates during the exposure period. The substantially higher ^{14}C-activity recorded for fructose is to be expected since this monosaccharide was used at a specific activity that was about 4-fold greater than the other sugars.

Table 10. Total ^{14}C-activity of filter papers placed in close proximity to seeds during their exposure to ^{14}C-labeled sugars

^{14}C-labeled sugar	Total activity (cpm · 10^3)[a]
Fructose	182.48
Galactose	82.25
Glucose	58.15
Sucrose	31.08

[a]Counts per minute to be multiplied by 1000.

^{14}C-Activity in ethanol soluble fraction

The total amount of radioactivity of the ethanolic extracts of the various seedling organs immediately after exposure and thereafter at 24 and 48 hours are summarized in Table 11. Radioactivity was observed to decrease steadily in the cotyledon. This was true for each ^{14}C-labeled sugar. On the other hand the radioactivity of the

Table 11. The amount of radioactivity (cpm · 10^4)[a] of the ethanolic extracts of the various seedling organs immediately after exposure to ^{14}C-sugars and thereafter at 24 and 48 hours

Time after exposure (hrs)	^{14}C-Sugar	Cotyledon	Embryonic axis	Hypocotyl	Radicle	Total
0	Fructose	168.81	3.84	-[b]	-[b]	172.64
	Glucose	107.54	2.46	-[b]	-[b]	110.01
	Galactose	84.06	2.23	-[b]	-[b]	86.30
	Sucrose	54.67	2.60	-[b]	-[b]	57.27
24	Fructose	107.41	29.72	-[b]	-[b]	137.13
	Glucose	55.02	17.92	-[b]	-[b]	72.94
	Galactose	57.17	16.43	-[b]	-[b]	73.60
	Sucrose	35.78	9.39	-[b]	-[b]	45.17
48	Fructose	34.85	-[b]	33.80	14.92	83.57
	Glucose	25.31	-[b]	15.70	6.33	47.33
	Galactose	6.74	-[b]	19.11	8.55	34.40
	Sucrose	17.56	-[b]	11.59	4.60	33.75

[a]Counts per minute to be multiplied by 10,000.

[b]Only at 48 hours after exposure could the embryonic axis be dissected into hypocotyl and radicle.

seedling organs increased with time and with the exception of glucose and sucrose, there was more radioactivity in the ethanolic extracts of the hypocotyl and radicle at 48 hours following exposure than in the cotyledons. The radioactivity of the embryonic axis increased 7-fold 24 hours after exposure of cotyledons to ^{14}C-fructose, glucose

and galactose. When cotyledons were exposed to ^{14}C-sucrose, radioactivity of the ethanolic extract of the embryonic axis had increased by 4-fold 24 hours following the exposure.

Fructose-U-^{14}C

The metabolic fate of fructose-U-^{14}C in the cotyledons immediately after its exposure to these organs and at 24 and 48 hours thereafter, is summarized in Table 12. A radioautogram of an 80% (v/v) ethanol extract of the cotyledons and embryonic axes following exposure of

Table 12. Activity of ^{14}C-sugars of cotyledons (cpm · 10^3)[a] and the percentage distribution of these sugars determined immediately after exposure to fructose-U-^{14}C (0.05 M) and thereafter at 24 and 48 hours

	Time (hr) following exposure					
	0		24		48	
	cpm	%	cpm	%	cpm	%
Immobile fraction	101.00	6.29	28.30	2.31	33.09	12.88
Stachyose	53.00	3.31	23.15	2.38	NDC	–
Compound A	37.44	2.35	NDC	–	NDC	–
Raffinose	NDC	–	18.00	1.85	NDC	–
Sucrose	1382.51	86.27	864.91	88.85	195.59	76.85
Glucose	NDC	–	NDC	–	NDC	–
Fructose	NDC	–	NDC	–	NDC	–
Compounds B and C	28.22	1.77	39.09	4.02	26.74	10.28

NDC: Not detectable on chromatogram.

[a]Counts per minute to be multipled by 1000.

cotyledons to ^{14}C-labeled sugars is presented in Fig. 13. ^{14}C-fructose was almost entirely converted to ^{14}C-labeled sucrose in the cotyledons immediately after exposure. This situation continued thereafter at 24 and 48 hours. Immediately after the exposure period and thereafter at 24 and 48 hours ^{14}C-sucrose accounted for 86.27, 88.85 and 76.85% respectively of all the ^{14}C-ethanol soluble carbohydrates resolved on a chromatogram. An interesting finding was that no hexoses were present in the ethanolic extracts of cotyledons exposed to ^{14}C-fructose. ^{14}C-labeled stachyose, and polymers which did not migrate on the chromatography paper after three ascents were also observed immediately after exposure and thereafter at 24 and 48 hours. These polymers will hereafter be referred to as the immobile fraction. An unidentifiable compound (compound A), which appeared as a dark spot and migrated faster than stachyose but slower than raffinose was noticed immediately after the exposure period. This compound had a mobility relative to fructose (R_{Fru}) of 0.16. Two other compounds were located on the chromatogram of a cotyledonary extract immediately following exposure, and thereafter at 24 and 48 hours. These compounds are referred to as compound B and compound C and are characterized by (R_{Fru}) values of 1.15 and 1.18 respectively. The activity of these two compounds increased from 1.77% of the total ^{14}C-activity on the chromatogram immediately following exposure, to 4.02 and 10.28% at 24 and 48 hours thereafter, respectively.

The data presented in Table 13 demonstrate that ^{14}C from fructose-U-^{14}C was translocated from the cotyledonary tissues to those of the embryonic axis of the soybean seedling during exposure. The rate of

Fig. 13. Picture of a radioautograph of 80% (v/v) ethanolic extract of cotyledons and embryonic axes of germinating soybeans fed various ^{14}C-sugars.

Std. Mix.: A mixture of reference sugars containing D-fructose, D-glucose, maltose, melibiose, raffinose, stachyose, and sucrose.

1 and 2: Cotyledons of seedlings sampled 24 hours after exposure of cotyledons to D-fructose-U-^{14}C and D-glucose-U-^{14}C respectively.

3 and 4: Embryonic axes of seedlings sampled 24 hours after exposure of cotyledons to D-fructose-U-^{14}C and D-glucose-U-^{14}C respectively.

5: Embryonic axes of seedlings immediately after exposure of cotyledons to D-glucose-U-^{14}C.

6: Cotyledons of seedlings immediately after exposure of these organs to D-galactose-U-^{14}C.

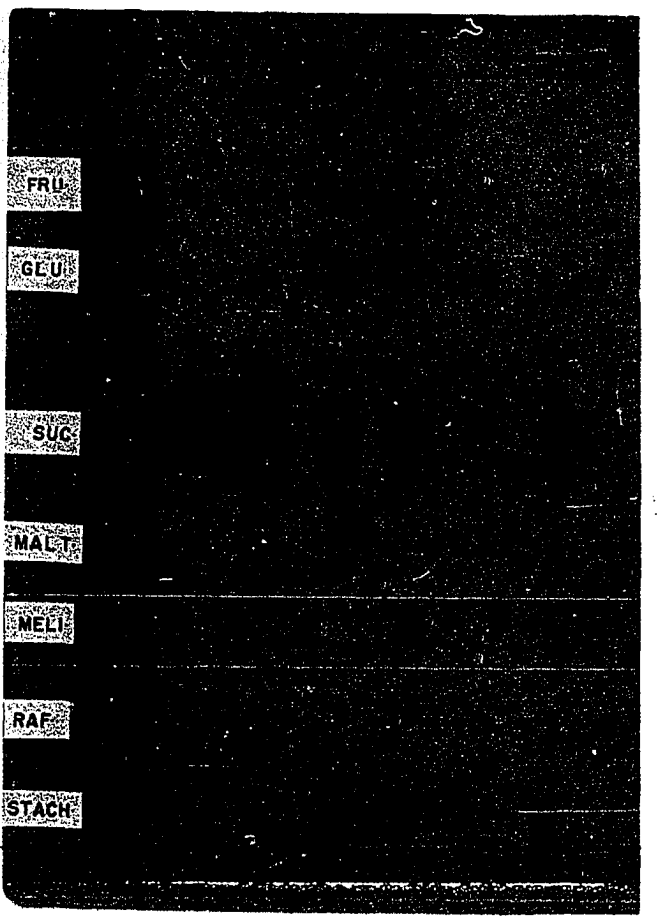

FRU
GLU
SUC
MALT
MELI
RAF
STACH

Table 13. Activity of ^{14}C-sugars of seedling organs (cpm · 10^3)[a] and the percentage distribution of these sugars determined immediately after exposure to ^{14}C-U-fructose (0.05 M) and thereafter at 24 and 48 hours

	Time (hr) following exposure							
	0		24		48			
	Embryonic axes				Hypocotyl		Radicle	
	cpm	%	cpm	%	cpm	%	cpm	%
Immobile fraction	2.93	7.10	5.96	2.43	14.65	5.10	8.95	9.72
Stachyose	2.70	7.75	18.54	7.18	NDC	–	NDC	–
Raffinose	NDC	–	20.18	8.14	36.55	12.65	17.23	18.78
Sucrose	30.89	85.14	63.32	25.69	70.73	24.47	14.63	16.00
Glucose	NDC	–	43.20	17.35	64.48	22.31	15.76	17.12
Fructose	NDC	–	79.36	32.00	78.31	27.10	15.03	16.38
Compound B	NDC	–	9.46	3.83	24.26	8.38	20.37	22.20
Compound C	NDC	–	7.80	3.15	NDC	–	NDC	–

NDC: Not detectable on chromatogram.

[a]Counts per minute to be multiplied by 1000.

transfer of ^{14}C to the growing tissues increased with time. As in the cotyledons, the embryonic axis contained 85% of its total ^{14}C in the form of radioactive sucrose immediately after exposure of the cotyledons to ^{14}C-fructose. Polymers which were located at the origin (immobile fraction), and stachyose were also detected. The total ^{14}C-activity of the ethanolic extract from the embryonic axes had increased 7 and 12-fold 24 and 48 hours following exposure respectively. This increase occurred at the rate of 10 cpm/hr.

A chromatogram of the ^{14}C-sugars in the embryonic axes at 24 hours following exposure revealed the presence of polymers (immobile fraction), and other compounds with R_{Fru} values similar to those of stachyose, raffinose, sucrose, glucose, fructose, and compounds B and C. The ^{14}C-activities of raffinose and stachyose were similar as were those of compounds B and C. The immobile fraction constituted 2.43% of the total ^{14}C-activity of all the resolvable compounds in the ethanol extract of the embryonic axis 24 hours following exposure to fructose-U-^{14}C. The total ^{14}C-activity of the hypocotyl exceeded that of the radicle by a factor of 2.25. This indicates a greater demand for metabolic substrates by the hypocotyl than by the radicle. Fructose was the predominant sugar in the hypocotyl and exceeded the levels of glucose, sucrose, and raffinose by 5, 3, and 12% respectively. Fructose, glucose, sucrose, and raffinose were present in the radicle in essentially the same amounts and together they constituted 70% of the total ^{14}C-sugars in this organ. Compound B was the predominant sugar and polymers larger than stachyose were also found in the radicle of seedlings 48 hours following exposure of cotyledons to fructose-U-^{14}C. These compounds accounted for 22 and 10% respectively of the total ^{14}C-sugars of the radicle.

Table 14 shows the translocation of ^{14}C-labeled sugars from the cotyledons to the growing seedling organs. Virtually all of the ^{14}C-activity taken up after 4 hours of exposure to fructose-U-^{14}C remained in the cotyledons. However, at 24 and 48 hours following exposure the cotyledons accounted for 78 and 42% respectively of the total ethanol soluble ^{14}C in the seedling. The marked increase of

Table 14. Distribution of ^{14}C-activity among seedling organs immediately after exposure to fructose-U-^{14}C (0.05 M) and thereafter at 24 and 48 hours

Time (hr)	Cotyledon %	Embryonic axis	Hypocotyl	Radicle
0	97.78	2.22	-[a]	-[a]
24	78.37	21.64	-[a]	-[a]
48	41.62	-[a]	40.52	17.86

[a]Only at 48 hours after exposure could the embryonic axis be dissected into hypocotyl and radicle.

^{14}C in the embryonic axis from 2 to 22% at 0 and 24 hours after exposure respectively, demonstrates that ^{14}C was rapidly translocated from the cotyledons to the growing tissues. This was further substantiated by the ^{14}C-activities of the hypocotyl and radicle 48 hours following exposure to ^{14}C-fructose. At this time these organs together accounted for 58% of the total ethanol soluble ^{14}C-activity in the entire seedling.

Glucose-U-^{14}C

Table 15 shows the uptake and interconversion of glucose-U-^{14}C by cotyledons immediately after exposure and thereafter at 24 and 48 hours. No ^{14}C-labeled hexoses could be detected in the cotyledons immediately after the exposure period, and during the ensuing 48 hours. ^{14}C-sucrose was the predominant labeled sugar in the cotyledons throughout the experimental period and accounted for 82, 90 and 86% of the total ^{14}C-sugars at 0, 24 and 48 hours after exposure respectively. Polymers which did

Table 15. Activity of ^{14}C-sugars of cotyledons (cpm · 10^3)[a] and the percentage distribution of these sugars determined immediately after exposure to glucose-U-^{14}C (0.05 M) and thereafter at 24 and 48 hours

	Time (hr) following exposure					
	0		24		48	
	cpm	%	cpm	%	cpm	%
Immobile fraction	65.06	5.97	13.25	2.42	14.35	7.10
Stachyose	46.61	4.29	NDC	–	NDC	–
Compound A	21.70	2.00	22.28	4.05	NDC	–
Raffinose	NDC	–	10.36	1.88	NDC	–
Sucrose	889.04	82.09	493.21	89.84	174.23	86.38
Glucose	NDC	–	NDC	–	NDC	–
Fructose	NDC	–	NDC	–	NDC	–
Compound B	32.55	3.00	9.95	1.81	13.53	6.54
Compound C	28.96	2.65	NDC	–	–	–

NDC: Not detectable on chromatogram.

[a]Counts per minute to be multiplied by 1000.

not migrate from the origin (immobile fraction), after three ascents, stachyose, and compounds A, B, and C were also present in the cotyledonary tissues immediately after exposure to ^{14}C-glucose.

The ^{14}C-activity of the immobile fraction increased at 24 and 48 hours following exposure. Compound A persisted in the cotyledonary tissues until 24 hours after exposure. Stachyose and compound C could not be detected at 24 and 48 hours; but raffinose was present in the cotyledonary tissues 24 hours following exposure. The only ^{14}C-sugars

observed in the cotyledonary tissues 48 hours after exposure of these tissues to glucose-U-^{14}C were the immobile fraction, stachyose, sucrose, and compound B.

The ^{14}C-sugars and the percentage distributions in the various seedling organs after exposure to glucose-U-^{14}C are shown in Table 16, and Fig. 13. ^{14}C-sucrose constituted 60 and 36% of the total

Table 16. Activity of ^{14}C-sugars of seedling organs (cpm · 10^3)[a] and the percentage distribution of these sugars determined immediately after exposure to ^{14}C-U-glucose (0.05 M) and thereafter at 24 and 48 hours

	Time (hr) following exposure							
	0		24		48			
	Embryonic axes				Hypocotyl		Radicle	
	cpm	%	cpm	%	cpm	%	cpm	%
Immobile fraction	NDC	–	NDC	–	6.94	5.11	4.19	6.11
Stachyose	NDC	–	8.82	5.95	6.64	4.87	3.75	7.96
Compound A	3.46	15.61	NDC	–	NDC	–	NDC	–
Raffinose	NDC	–	19.08	12.93	10.95	7.80	8.78	18.63
Sucrose	13.38	60.44	51.91	35.45	36.99	26.94	12.30	26.09
Glucose	4.01	17.94	24.72	16.79	26.54	19.38	5.03	10.66
Fructose	NDC	–	42.41	28.90	37.76	27.48	6.78	14.39
Compound C	NDC	–	NDC	–	11.58	8.44	6.31	13.38

NDC: Not detectable on chromatogram.

[a]Counts per minute to be multiplied by 1000.

^{14}C-labeled sugars in the embryonic axes immediately after exposure of cotyledons to glucose-U-^{14}C and thereafter, at 24 hours. ^{14}C-glucose was present in the embryonic axes at 0 and 24 hours after exposure and accounted for 18 and 17% of the total labeled sugars in these tissues respectively. Compound A was the only other sugar present in the embryonic axes at 0 hour after exposure but it was not detected in these tissues 24 hours later. ^{14}C-stachyose, raffinose, sucrose, glucose, and fructose were present in the embryonic axes at 48 hours with sucrose being the predominantly ^{14}C-active sugar. At 48 hours, the hypocotyl contained ^{14}C-stachyose, raffinose, sucrose, glucose, fructose, compound C and an immobile fraction. These sugars were also present in the radicle at 48 hours but the ^{14}C-activity of each sugar was lower in these tissues than in the hypocotyl.

Table 17 illustrates the movement of ^{14}C (from glucose-U-^{14}C) out of the cotyledons and into the growing tissues of the germinating

Table 17. Distribution of ^{14}C-activity among seedling organs immediately after exposure of cotyledons to glucose-U-^{14}C (0.05 M) and thereafter at 24 and 48 hours

Time (hr)	Cotyledon %	Embryonic axis %	Hypocotyl %	Radicle %
0	97.76	2.24	-[a]	-[a]
24	75.47	24.54	-[a]	-[a]
48	53.28	-[a]	33.30	13.42

[a]Only at 48 hours after exposure could the embryonic axis be dissected into hypocotyl and radicle.

seedling. Total ^{14}C-labeled sugars in the cotyledons decreased by approximately 50%, 48 hours after these tissues were exposed to glucose-U-^{14}C. At the same time the hypocotyl and radicle accounted for 33 and 13% respectively of the ^{14}C-labeled sugars in the whole seedling. These results demonstrate the translocation of ^{14}C from the cotyledons and into the growing tissues. Also apparent is the increased rate of movement of metabolites from the cotyledonary tissues to the growing tissues as germination progressed.

Galactose-U-^{14}C

The carbohydrates formed in the cotyledonary tissues subsequent to the uptake of galactose-U-^{14}C by these tissues are shown in Table 18 and Fig. 13. ^{14}C-Sucrose constituted over 80% of the total ^{14}C-sugars from the cotyledons of germinating soybeans immediately after exposure of these organs to galactose-U-^{14}C and thereafter at 24 and 48 hours. In addition to ^{14}C-sucrose, several other ^{14}C-sugars were present in the cotyledonary tissues immediately after exposure. These sugars were stachyose, raffinose, compounds A, B and C, and an immobile fraction. Stachyose and compounds A and C were not observed 24 hours after exposure and the only other ^{14}C-sugar present with sucrose at 48 hours was compound B.

The ^{14}C-carbohydrates and their percentage distributions in the embryonic axes, hypocotyl and radicle are shown in Table 19. ^{14}C-Sucrose was the only carbohydrate observed in the embryonic axes immediately following exposure of the cotyledons to galactose-U-^{14}C. This sugar was present in the embryonic axes 24 hours later in substantial

Table 18. Activity of ^{14}C-sugars of cotyledons (cpm · 10^3)[a] and the percentage distribution of these sugars determined immediately after exposure to galactose-U-^{14}C (0.05 M) and thereafter at 24 and 48 hours

	Time (hr) following exposure					
	0		24		48	
	cpm	%	cpm	%	cpm	%
Immobile fraction	46.20	5.03	21.28	3.96	NDC	–
Stachyose	36.11	3.96	NDC	–	NDC	–
Compound A	24.79	2.90	NDC	–	NDC	–
Raffinose	21.21	2.29	50.65	9.57	NDC	–
Sucrose	692.03	82.16	441.95	83.16	45.43	91.27
Compound B	11.43	1.22	17.57	3.30	4.12	8.73
Compound C	17.35	1.98	NDC	–	NDC	–

NDC: Not detectable on chromatogram.

[a]Counts per minute to be multiplied by 1000.

amounts. At this time, polymers which remained at the origin after three chromatographic ascents (immobile fraction), raffinose, glucose, fructose and compound B were also present. ^{14}C-Raffinose and fructose contained the highest activities, followed by sucrose, glucose, compound B and the immobile fraction.

The hypocotyl contained about equal amounts of ^{14}C-fructose, glucose and sucrose at 48 hours. Also present in this organ at this time were an immobile fraction, stachyose and compound B, which together accounted for 29% of the total ^{14}C-sugars resolved on a chromatogram. The radicle contained the bulk of its ^{14}C-sugars in the form of stachyose

Table 19. Activity of ^{14}C-sugars of seedling organs (cpm · 10^3)[a] and the percentage distribution of these sugars determined immediately after exposure to galactose-U-^{14}C (0.05 M) and thereafter at 24 and 48 hours

	Time (hr) following exposure							
	0		24		48			
	Embryonic axes				Hypocotyl		Radicle	
	cpm	%	cpm	%	cpm	%	cpm	%
Immobile fraction	NDC	–	6.43	4.28	5.08	3.07	3.59	6.12
Stachyose	NDC	–	NDC	–	31.76	19.13	20.19	34.41
Raffinose	NDC	–	38.90	26.01	NDC	–	–	–
Sucrose	19.42	100	34.16	22.78	38.87	23.31	12.18	20.62
Glucose	NDC	–	22.91	15.33	36.38	21.83	5.90	9.83
Fructose	NDC	–	40.05	26.77	43.87	26.36	7.73	13.09
Compound B	NDC	–	7.28	4.85	10.47	6.31	9.30	15.95

NDC: Not detectable on chromatogram.

[a]Counts per minute to be multiplied by 1000.

which constituted 34% of the total ^{14}C-sugars. ^{14}C-Sucrose, fructose, compound B, glucose and an immobile fraction were also present in the tissues of the radicle and together comprised the remaining 66%.

Table 20 summarizes the rate of transfer of ^{14}C (from galactose-U-^{14}C) from the cotyledons to the embryonic organs of the germinating soybean seedling. It is clear from these data that metabolites necessary for growth are transported from the energy source (cotyledons) to the energy sink (embryonic axis). As germination progressed the rate of translocation of metabolites from the cotyledons to the embryonic axis increased

Table 20. Distribution of ^{14}C-activity among seedling organs immediately after exposure of cotyledons to galactose-U-^{14}C (0.05 M) and thereafter at 24 and 48 hours

Time (hr)	Cotyledon %	Embryonic axis %	Hypocotyl %	Radicle %
0	97.34	2.66	-[a]	-[a]
24	79.14	20.86	-[a]	-[a]
48	18.00	-[a]	55.90	26.10

[a]Only at 48 hours after exposure could the embryonic axis be dissected into hypocotyl and radicle.

as is evidenced by a 21 and 82% loss of total ^{14}C-sugars from the cotyledons at 24 and 48 hours following exposure respectively.

Sucrose-U-^{14}C

The metabolic fate of ^{14}C-sucrose taken up by cotyledons is presented in Table 21. Immediately after the 4 hour exposure period, ^{14}C-stachyose and raffinose were present along with ^{14}C-sucrose in the cotyledons. These sugars accounted for 5, 6 and 89% respectively of the total ^{14}C-sugars. The only other labeled sugar, besides sucrose at 24 hours following the exposure, was compound C which accounted for only 3% of the total ^{14}C-sugars in the cotyledons. The ^{14}C-carbohydrates present in the cotyledons 48 hours after the exposure were stachyose, sucrose, glucose, fructose, and compound C. ^{14}C-Sucrose was the predominantly labeled sugar accounting for 81% of the total ^{14}C-activity.

Table 21. Activity of ^{14}C-sugars of cotyledons (cpm · 10^3)[a] and the percentage distribution of these sugars determined immediately after exposure to sucrose-U-^{14}C (0.05 M) and thereafter at 24 and 48 hours

	Time (hr) following exposure					
	0		24		48	
	cpm	%	cpm	%	cpm	%
Stachyose	23.83	4.55	NDC	–	8.14	4.89
Raffinose	31.27	5.98	NDC	–	NDC	–
Sucrose	467.88	89.46	280.83	97.20	133.68	81.43
Glucose	NDC	–	NDC	–	3.70	2.21
Fructose	NDC	–	NDC	–	10.77	6.10
Compound C	NDC	–	8.25	2.81	11.17	6.84

NDC: Not detectable on chromatogram.

[a]Counts per minute to be multiplied by 1000.

The ^{14}C-sugars present in the embryonic axis, hypocotyl and radicle, immediately after exposure of cotyledons to sucrose-U-^{14}C and thereafter at 24 and 48 hours, are presented in Table 22. ^{14}C-Sucrose was the only labeled carbohydrate present in the embryonic axes immediately after cotyledons were exposed to this sugar. The ^{14}C-sugars present in the embryonic axes at 24 hours were: fructose, sucrose, glucose and compound C in decreasing order of ^{14}C-activity. These same carbohydrates were present in the hypocotyl and radicle at 48 hours with sucrose having the highest ^{14}C-activity in both organs.

Table 22. Activity of ^{14}C-sugars of seedling organs (cpm · 10^3)[a] and the percentage distribution of these sugars determined immediately after exposure to sucrose-U-^{14}C (0.05 M) and thereafter at 24 and 48 hours

	Time (hr) following exposure							
	0		24		48			
	Embryonic axes				Hypocotyl		Radicle	
	cpm	%	cpm	%	cpm	%	cpm	%
Sucrose	24.94	100	23.61	34.69	28.41	33.40	6.93	34.22
Glucose	NDC	–	13.66	20.10	22.16	26.10	3.88	18.91
Fructose	NDC	–	24.86	36.62	26.05	30.83	4.55	22.03
Compound C	NDC	–	5.80	8.60	8.59	9.68	5.16	24.85

NDC: Not detectable on chromatogram.

[a]Counts per minute to be multiplied by 1000.

Table 23 summarizes the rate of transfer of ^{14}C (derived from sucrose-U-^{14}C) from the cotyledons to the embryonic axes, hypocotyl

Table 23. Distribution of ^{14}C-activity among seedling organs immediately after exposure of cotyledons to sucrose-U-^{14}C (0.05 M) and thereafter at 24 and 48 hours

Time (hr)	Cotyledon %	Embryonic axis %	Hypocotyl %	Radicle %
0	95.45	4.55	-[a]	-[a]
24	79.17	20.84	-[a]	-[a]
48	52.07	-[a]	34.31	13.63

[a]Only at 48 hours after exposure could the embryonic axis be dissected into hypocotyl and radicle.

and radicle. These results indicate that translocation of ^{14}C from the site of uptake to the embryonic axis occurred during the exposure, and that after the cotyledons were exposed to sucrose-U-^{14}C there was a transfer of ^{14}C out of the cotyledons and into the growing embryonic tissues.

DISCUSSION

The objectives of these investigations were: to determine the pattern of carbohydrate change in the various seed/seedling organs, to study the metabolic fates of exogenously administered ^{14}C-sugars in imbibed seeds, and to monitor the changes in water content and dry matter during germination of the soybean. Seed germination is a complex process, the onset of which is characterized by: seed hydration, increased respiratory and enzymatic activities, enzyme synthesis de novo, mobilization and interconversion of stored food reserves, and the translocation of these reserves from storage to embryonic axes. Because of the absolute requirement of adequate moisture for the onset of germination and the mobilization and transfer of stored materials from the storage to the growing tissues the results of these studies will be discussed in a concerted manner. For the purpose of simplicity the ungerminated seed will be discussed separately and the germination period studied will be discussed in two sections. Section one will pertain to the first 60 hours of germination, and section two will cover 66 through 192 hours. The discussion following this will be devoted to the metabolic fates of the ^{14}C-sugars which were administered to the cotyledons of germinating soybeans.

The Ungerminated Seed

The low moisture content of the dry seed (Table 2), and the extremely low levels of free hexoses and high levels of oligosaccharides in the cotyledons, embryonic axis (Tables 4 and 5), and seed coats

respectively, are indicative of a resting organism. Seeds are an important means of species perpetuation and by necessity exhibit a low metabolic activity thereby conserving their supply of stored energy reserves. When favorable conditions prevail these stored reserves are utilized for germination and early seedling growth. In these studies non-reducing carbohydrates comprised 96% of the total carbohydrates (80% ethanol soluble carbohydrates) in the whole seed. In previous studies on soybean germination respiratory activity of the viable ungerminated seed was reported to be negligible (Wahab and Burris, 1971) and virtually all of the water and ethanol soluble carbohydrates were in the form of non-reducing sugars (Abrahamsen and Sudia, 1966; Pazur et al., 1962; and Wahab and Burris, 1971).
The moisture contents of the seed coats, cotyledons and embryonic axis were the same (4%), indicating an equilibrium condition between these seed entities. However, the molar concentrations of stachyose and raffinose in the embryonic axis of the dry seed were 7 and 5 times greater respectively than the concentrations of these sugars in the cotyledons of the dry seed. This indicates the existence in the dry seed of a concentration gradient between the oligosaccharides of the cotyledons and embryonic axis. This situation was further exemplified by the ethanol soluble carbohydrates of the various seed entities. Ethanol soluble sugars constituted 25% of the dry weight of the embryonic axis (Table 5), whereas in the cotyledons and seed coats these carbohydrates accounted for 9% (Table 4) and 1% respectively of the dry weight of these tissues. The substantially higher percentage of total carbohydrates within the embryonic axis is tenable since this

organ becomes physiologically active faster than the cotyledons following imbibition (Abrahamsen and Sudia, 1966; Wahab and Burris, 1971). Despite this large percentage of ethanol soluble sugars in the embryonic axis, this organ accounts for only 3% of the total dry weight of the seed, and therefore, contains only 7% of the total ethanol soluble carbohydrates in the entire dry seed. It is interesting that ethanol soluble sugars were present in the seed coats of dry seeds (Fig. 5). Such findings have not been hitherto reported and an explanation for their possible role in germination will be offered in the following section.

The small amounts of free hexoses in the cotyledons of ungerminated seed is to be expected since these sugars serve as immediate respiratory substrates in living organisms. The small amounts of maltose in the cotyledons of the unimbibed seeds could be attributed to a natural residual by-product following seed maturation. This disaccharide was reported to be the most abundant sugar in the soybean plant at the time of flowering and pod formation (Quillet and Bourdon, 1956). Maltose was present in wheat flour (Koch et al., 1951) and Reynolds et al. (1958) suggested that this sugar serves as a respiratory substrate during germination. Maltose has been shown by Nigam and Giri (1960) and Chen and Varner (1969) to be converted to glucose, maltotriose, maltotetraose, and sucrose during germination of bean and cereal seeds.

Germination from 0 through 60 hours

Both the dry weight and the moisture content gave an index of the rate of movement of food reserves from the cotyledons to the embryonic

axis. With the exception of the rapid initial water uptake of the cotyledons during the first 24 hours of imbibition (Fig. 1), the change in water content during the following 36 hours was inversely proportional to the dry weight (Fig. 2). A similar relationship between these two factors was found in sunflower (Miller, 1910) and wheat (Yocum, 1925). Embryonic axes increased in water content in an exponential manner (Fig. 1). At 60 hours, there was 1100 times more water in the tissues of the embryonic axes than at the commencement of imbibition. The change in water content of the embryonic axes was highly correlated with the change in dry matter of these tissues. Also, the change in dry matter of the embryonic axis from 24 through 60 hours was inversely proportional to the change in dry matter of the cotyledons during this period. This is indicative of the transfer of material from the cotyledons to the embryonic axes, which became apparent 24 hours following imbibition.

Although the marked decline in total carbohydrates of the seed coats at 24 hours (60%) was insignificant compared to the corresponding decline observed in the embryonic axes and cotyledons at this time, these carbohydrates may be important as respiratory substrates immediately following imbibition. The absence of free hexoses in the seed coats at 24 hours and the accompanying decrease of sucrose and stachyose may be attributed to: (1) the utilization of these sugars by the cotyledons, (2) the digestion of these sugars by microflora present on the seed coats, and (3) the leaching of these sugars from the seed coats to the germination substrate.

The rapid change in physiological status of the seed following imbibition is demonstrated by the carbohydrate changes in the cotyledon and embryonic axis just 6 hours following imbibition (Figs. 6, 7 and 8 and Tables 4 and 5). There was an 8% decline in the level of total ethanol soluble carbohydrates within the cotyledons 6 hours following imbibition. This decline continued throughout the germination period and at 48 hours, total ethanol soluble carbohydrates had been depleted by 50%. The contents of stachyose, raffinose, and sucrose in the cotyledons had decreased even more markedly during the first 48 hours of germination. These sugars had decreased to 17, 32, and 43% respectively, of their initial levels 48 hours after imbibition. Since raffinose and sucrose are immediate hydrolysis products of stachyose and raffinose respectively, it is apparent from these data that raffinose and sucrose are utilized and regenerated simultaneously during the germination of the soybean. The operation of such a metabolic system can be envisaged by the presence of α-galactosidases in the germinating soybean (Pazur et al., 1962).

The significant increases in stachyose, raffinose and sucrose observed at 18 hours could be attributed to the de novo synthesis of these oligosaccharides in the germinating soybean. Dupéron (1964) demonstrated such synthesis of oligosaccharides in Phaesolus vulgaris seeds from ^{14}C-glucose. Results of the ^{14}C-labeled experiments herein reported also demonstrated the de novo synthesis of sucrose, raffinose and stachyose in germinating soybeans. Furthermore, the presence of enzymes and cofactors necessary for the conversion of free glucose and fructose to sucrose, raffinose and stachyose have been reported in

several other seed species during germination (Bourne et al., 1965; Lehle et al., 1970; Pridham and Hassid, 1965; Shiroya, 1963; Tanner and Kandler, 1966, 1968). The source of glucose for the presumed de novo synthesis of sucrose, raffinose, and stachyose at 18 hours may be starch or starch-like compounds which, according to Von Ohlen (1931) are present in the cotyledons of the mature soybean.

The variable levels of melibiose observed in the cotyledons during germination might have been due to invertase hydrolysis of raffinose. Melibiose thus formed might have been subjected to α-galactosidase hydrolysis to yield galactose and glucose. One conspicuous feature of the carbohydrates in the germinating soybean is the absence of D-galactose in the ethanolic extracts throughout the germination period. The possibility that this sugar is rapidly converted into another carbohydrate during the extraction step can be safely ruled out because tissues were ground in boiling 80% ethanol and the filtrate was concentrated under vacuum at 50°C for periods of 30 to 40 minutes. The absence of galactose in germinating soybeans was also reported by Abrahamsen and Sudia (1966) and Pazur et al. (1962). Shiroya (1963) studying the metabolism of raffinose in germinating cotton also reported the absence of galactose in the seedlings. Pazur and co-workers studying the metabolism of galactose, succeeded in isolating several enzymes from the germinating soybean that participate in the metabolism of galactose. These workers suggested that D-galactose was rapidly utilized as soon as it was liberated from raffinose and stachyose. Shiroya studying the fate of ^{14}C-galactose in cotton seeds similarly reported that there was a

highly efficient galactose utilizing system in the cotyledons which converted this sugar into other compounds. Other workers studying the fate of galactose in several seed species reported the conversion of this hexose into cell wall polysaccharides (Altermatt and Neish, 1956; Ordin and Bonner, 1957; Roberts and Butt, 1969; Thimann et al., 1958).

The dry weight loss of the embryonic axis at 12 hours (Table 3) and the fairly substantial loss in total ethanol soluble carbohydrates occurring at this time (Table 5), suggest the onset of germination (Mayer and Poljakoff-Mayber, 1966, p. 102). These data also suggest that the embryonic axis becomes physiologically active earlier than the cotyledons. Further evidence of this was presented by Abrahamsen and Sudia (1966) who demonstrated a substantial loss in total soluble carbohydrates in the embryonic axis during the first 24 hours of germination, but not in the cotyledon. Also, Wahab and Burris (1971) showed that embryonic axis of soybean respired 7 and 12 times faster than cotyledons at 12 and 24 hours following imbibition.

The dry matter gain and carbohydrate contents of the embryonic axis during the first 60 hours of germination were inversely proportional to the dry weight and carbohydrate levels respectively of the cotyledons (Tables 3, 4, and 5 and Figs. 2, 6, 7, and 8). Stachyose decreased markedly in the embryonic axis, raffinose was variable, and sucrose increased by about 100% at 60 hours. Free glucose and fructose levels increased in the cotyledon as germination progressed. These hexoses were highly correlated with the water content and dry matter loss of the cotyledonary tissues during the first 60 hours of germination.

Glucose and fructose could not be detected in the embryonic axis of the dry seed (Fig. 5), but together these hexoses constituted 85% of the total carbohydrates of the embryonic axis at 60 hours. These results demonstrate that as total sugars are depleted in the cotyledon they increase in the embryonic axis. Also, carbohydrates in the embryonic axis of the germinating soybean are predominantly reducing while in the cotyledonary tissues they are mostly non-reducing sugars. This situation may be attributed to the demand for readily utilizable substrates such as fructose and glucose by the growing seedling. These substrates are essential for respiration, for synthesis of structural materials, amino acids, protein and nucleic acids.

Germination from 66 through 192 hours

Total carbohydrates of the cotyledons continued to decline during the period 66 through 96 hours but at a much slower rate than was observed from 0 through 60 hours. The total carbohydrate levels of the radicle, hypocotyl and apical meristem (Tables 6, 7, and 8) were highly correlated with the loss in total sugars of the cotyledons. This indicates that sugars are rapidly transferred from the cotyledons to the anabolic organs for growth processes. Levels of total carbohydrates in the hypocotyl exceeded those of the cotyledon from 66 hours through the end of the experimental period. Glucose and fructose constituted the largest portion of these sugars ranging from 78 to 100% of the total ethanol soluble carbohydrates in the hypocotyl. Also, the molar concentrations of fructose and glucose were 10-fold greater in the hypocotyl than in the cotyledon. These results are

consistent with those of the embryonic axis indicating that soluble sugars are present mainly in their reducing form in tissues of the growing seedling. As germination progressed the levels of stachyose and raffinose decreased to undetectable amounts in the cotyledons. These oligosaccharides were detected in the tissues of the growing seedling to a limited extent and their presence might have been due to their initial levels observed in the embryonic axis of the dry seed.

Throughout the experimental period sucrose was the most abundant sugar in the cotyledons. This may be indicative of the importance of sucrose in maintaining a desirable osmotic potential within the cotyledons. Also, more negative free energy is conserved in the sucrose molecule than in the constituent hexose moieties. This is because of the energy liberated during hydrolysis of the carbon-oxygen-carbon bond between the fructosyl and glucosyl moieties of sucrose.

A marked increase in total carbohydrates and a parallel increase in reducing sugars were observed in the apical meristem as this organ developed (Table 7). This was accompanied by a corresponding decrease in the level of reducing sugars within the hypocotyl and a noticeable increase in the level of total and non-reducing carbohydrates within the cotyledons at 120 hours. These results indicate that as the apical meristem developed an immediate demand was placed on the hypocotyl for metabolic substrates. Thus, the hypocotyl was functioning essentially as a source of energy at this time rather than as an energy sink. The increase in nonreducing sugar levels of the cotyledons between 96 and 120 hours demonstrates the ability of the cotyledons to mobilize and transfer its stored energy reserves to the anabolic

organs as the demand for metabolic substrates increases. The source of increased total carbohydrate and sucrose levels in the cotyledons at 120 hours could be lipids. The free fatty acids of a lipid molecule can undergo β-oxidation to yield acetate. Acetate can be converted to malate via the glyoxylate cycle and malate can be metabolized to glucose by reversal of glycolysis. The enzymes of the glyoxylate cycle are reported to be present in the germinating soybean, and attain maximal activities about the fifth day following imbibition (Abrahamsen and Sudia, 1966; Carpenter and Beevers, 1959; and Yamamoto and Beevers, 1960).

The radicle increased steadily in dry weight (Table 3), but at a slower rate compared to the hypocotyl and apical meristem. The carbohydrate levels of the radicle remained essentially constant from 66 through 192 hours (Table 8) and as in the case of the hypocotyl and apical meristem, glucose and fructose constituted the major portion of these sugars. However, fructose levels were consistently greater than glucose levels throughout the germination period. Similar results were reported by Maclachlan et al. (1970) who studied the regulation of sugar levels in the pea epicotyl. They reported that fructose was the predominant sugar in meristematic regions of the pea epicotyl, while glucose was barely detectable. These workers further reported that the distribution of invertase activity was parallel to that of glucose, and sucrose synthetase was most active in regions where fructose and sucrose were concentrated. Maclachlan and co-workers suggested therefore, that the principal factors controlling sugar levels in the pea epicotyl are location and relative activities of

invertase and sucrose synthetase in the epicotyl. The greater proportion of sugars in the aerial tissues (hypocotyl, epicotyl, unifoliate leaves, and shoot apex), and the greater dry matter of these organs relative to the roots, suggest that the aerial tissues impose a higher demand for energy reserves from the cotyledons than do the roots.

Results of the water content, dry matter and carbohydrate levels of the entire seedling (Tables 2, 3, and 9) substantiate the claim made earlier in the introduction that studies based on the whole seed/seedling obscure the changes occurring in the embryo or embryonic parts. Such studies provide no meaningful physiological insights into germination and consequently, do not contribute towards the elucidation of germination from a metabolic point of view.

A comparison of the data for each seedling organ with those of the entire seedling indicates that the hypocotyl probably plays the biggest role in the carbohydrate aspect of early seedling physiology. Tissues of the hypocotyl contained 40, 60, and 66% of the total carbohydrates, water, and dry matter respectively of the whole seedling at the end of the experimental period. The dry matter content of the hypocotyl was inversely proportional to that of the cotyledon from 66 through 192 hours, and these two organs contained essentially the same amount of dry matter at 192 hours.

The dry matter data presented in Table 3 indicates a 63% decrease in cotyledonary dry weight from 0 through 192 hours. However, 80% of this loss was accounted for by the increase in dry weight of the embryonic parts (hypocotyl, radicle, apical meristem) at 192 hours. Based on respiratory data of cotyledons during soybean germination (Wahab and

Burris, 1971) the loss in dry matter of the cotyledons due to respiration was calculated. Results of these calculations indicate that the portion of dry matter loss of the cotyledon not accounted for by the dry weight increase of the remaining seedling organs was due to respiratory losses. Thus, these results demonstrate the importance of the soybean cotyledon as storage tissues and the efficiency of this organ to mobilize and convert its stored energy reserves to utilizable substrates for germination and early seedling growth. This is further demonstrated by the total ethanol soluble carbohydrate contents of the cotyledon and embryonic parts at the end of the experimental period (Tables 4 and 9). At this time the embryonic parts of the seedling contained over 4 times as much ethanol soluble sugars than did the cotyledons, which had lost 90% of their stored soluble carbohydrates observed at the time of imbibition. Furthermore, 70% of the total ethanol soluble carbohydrates present in the whole seedling at 192 hours was in the form of reducing sugars, primarily glucose and fructose.

^{14}C-Uptake and Translocation Studies

The ^{14}C-activities of the filter papers (Table 10) suggest that each ^{14}C-sugar was incorporated and respired by the seedling tissues during the exposure period. The specific activity of ^{14}C-fructose was about 4-fold greater than that of the other sugars at the initiation of the experiment. Consequently the amount of radioactivity taken up by the cotyledonary tissues will be greater for ^{14}C-fructose than for the other sugars during the exposure period assuming that uptake rate is

not affected by the higher specific activity of the ^{14}C-fructose. When adjustments are made for the higher specific activity of ^{14}C-fructose it becomes apparent that this sugar and ^{14}C-glucose were taken up and respired to approximately the same extent during the exposure period. ^{14}C-Galactose was respired at a rate greater than that observed for the other ^{14}C-labeled sugars. ^{14}C-Sucrose on the other hand was respired at a rate lower than the other ^{14}C-labeled sugars. These results suggest that fructose, glucose, and galactose are preferred over sucrose as respiratory substrates and that more ^{14}C-galactose is consumed in respiration than the other ^{14}C-labeled sugars. Similar results were reported by Burstrom (1948), who postulated that galactose is mainly consumed in respiration instead of being utilized for synthetical purposes by wheat root tips. However, Ordin and Bonner (1957) reported that galactose was respired to CO_2 in oat coleoptile sections at rates lower than those for glucose. The data presented in Table 11 demonstrate that during the exposure period ^{14}C-metabolites were taken up by the cotyledons and translocated from these tissues to those of the embryonic axis. Furthermore, these data substantiate the generally held tenet that during seed germination food reserves are mobilized and translocated from the cotyledon to the growing tissues.

Although the specific activity of ^{14}C-sucrose was essentially the same as those of ^{14}C-glucose and ^{14}C-galactose, ^{14}C-sucrose was absorbed to the lowest extent by the cotyledons during the exposure period. This is in variance with the results of Kriedemann and Beevers (1967a), who reported that ^{14}C-labeled sucrose uptake exceeded those of

^{14}C-glucose, and ^{14}C-fructose by cotyledons of castor bean seedling. However, Maclachlan et al. (1970) reported that ^{14}C-sucrose was neither absorbed nor metabolized by pea epicotyl sections more rapidly than either hexose. With the exception of sucrose, the amount of ethanol soluble ^{14}C within the embryonic axis increased by approximately 7-fold for all the ^{14}C-labeled sugars at 24 hours following the exposure period. At 48 hours following the exposure period, the tissues of the embryonic axis (hypocotyl and radicle) had a greater amount of radioactivity than those of the cotyledon when fructose and galactose were the radioactive sugars. Also, there was a steady decline in the amount of radioactivity of the entire seedling following the labeling. This decrease in radioactivity of the ethanolic extract may be attributed to loss of ^{14}C as $^{14}CO_2$ via respiration, and to the assimilation of ^{14}C into ethanol insoluble compounds. Although no quantitative determinations were made of the radioactivity of the ethanol insoluble residues, they were, nevertheless, found to be radioactive. These results agree with those of Larsen and Beevers (1965), Ordin and Bonner (1957), Roberts and Butt (1969), and Thimann et al. (1958). In addition, Abdul-Baki (1969) reported that 50-70% of the total ^{14}C-glucose administered to barley during the early hours of germination appeared as $^{14}CO_2$. The rest of the incorporated label appeared in hemicellulose and starch, water-soluble ethanol-insoluble carbohydrates, and to a lesser extent in proteins and cellulose. McConnell (1957) also reported that 17% of the ^{14}C from ^{14}C-labeled wheat seeds was lost as $^{14}CO_2$ from respiration of the germinating seeds. The fates of the ^{14}C-labeled sugars following

exposure to cotyledons (Fig. 13 and Tables 12 through 22), clearly indicate that sucrose is the preferred form of carbohydrate storage in the cotyledons of soybeans and substantiates the results herein reported on carbohydrate metabolism. This sugar constituted at least 80% of the total ^{14}C-activity obtained from the resolvable sugars immediately following exposure to labeled fructose, glucose, galactose and sucrose and thereafter at 24 and 48 hours. The presence also of ^{14}C-labeled raffinose, stachyose and higher molecular weight carbohydrates (immobile fraction) in the cotyledonary tissues following exposure to ^{14}C-glucose, galactose, fructose and sucrose strongly suggests that the enzymes and cofactors which have been shown (Bourne et al., 1965; Lehle et al., 1970; Pridham and Hassid, 1965; Rast et al., 1963; Senser and Kandler, 1967; Tanner and Kandler, 1966; Tanret, 1913; Stumpf and Barber, 1957) to participate in the synthesis of these oligosaccharides in other seeds are also present in the germinating soybean. From classical metabolism, labeled hexose incorporated into plant tissues is phosphorylated by hexokinase to hexose-6-phosphate. After that it may be converted to hexose-1-phosphate, UDP-glucose, sucrose and polysaccharides. Investigators studying the fates of ^{14}C-sugars in plants reported the conversion of ^{14}C-glucose, galactose, fructose and sucrose into hexose monophosphates, UDP-glucose, sucrose, hemicellulose, myoinositol, cell wall polysaccharides, amino acids, organic acids and other unidentifiable ^{14}C-compounds (Abdul-Baki 1969; Edelman et al., 1959; Hassid and Putman, 1952; Kriedemann and Beevers, 1967a, b; Larsen and Beevers, 1965; Matheson and St. Clair, 1971; Mertz and Nordin, 1971). These workers concur that soon after

exposure of plant and seedling tissues to ^{14}C-labeled fructose, glucose, and galactose, there is a decrease in the amount of radioactivity in these sugars and a corresponding increase in radioactivity of compounds derived from these sugars.

Verbascose [O-α-D-galactopyranosyl-(1 → 6)-O-α-D-galactopyranosyl-(1 → 6)-O-α-D-galactopyranosyl-(1 → 6)-O-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside] can be formed by the addition of galactose to the terminal galactosyl moiety of stachyose at the six carbon position. Therefore it is speculated that the immobile fraction seen on chromatograms of ^{14}C-ethanolic extracts might consist of this oligosaccharide. It is also possible that the immobile fraction might consist of oligosaccharides such as maltotriose or maltotetraose. The synthesis of these two oligosaccharides have been shown in germinating green gram seeds by Nigam and Giri (1960). Compound A (R_{Fru} = 0.16) which was also noticed on chromatograms of ^{14}C-ethanolic extracts could conceivably be a hydrolysis product of stachyose in ash manna (French, 1954) called manninotriose. It is formed by invertase hydrolysis of stachyose and has the structural formula [O-α-D-Galactopyranosyl-(1 → 6)-O-α-D-galactopyranosyl-(1 → 6)-D-glucose]. Mannintriose bears the same relationship to stachyose as melibiose bears to raffinose, and migrates slightly faster than stachyose on a paper chromatogram prepared by Wild and French (1952). The unidentifiable compounds (B and C) might conceivably be intermediates of the glycolytic pathway or Krebs' cycle such as trioses or organic acids.

The most striking feature of these data (Tables 12 through 22) is the clear indication that, within any one seedling organ, the three supplied hexoses were equally available for uptake and conversion to other products. In particular the three hexoses were used equally for sucrose, raffinose and stachyose synthesis and interconverted to approximately the same extent.

SUMMARY AND CONCLUSIONS

The data presented herein generally support the hypotheses that (1) studies on the whole seed/seedling obscure the changes taking place within each morphological entity, (2) soluble carbohydrates should be fractionated and quantified in order to determine the contribution of each sugar to the carbohydrate metabolism of germinating soybeans, and (3) determinations must be carried out at closely spaced time intervals to further understand the physiological events which follow imbibition and seed germination.

It is obvious that the cotyledons function primarily as storage organs which supply the substrates necessary for growth of the embryonic axis. However, evidence was presented for the utilization of stored carbohydrates within the embryonic axis as growth of this organ commenced. Transfer of material from the cotyledons to the embryonic axis was not evident until after embryonic growth had commenced.

Another advantage of studying the seed/seedling organs separately was illustrated by the presence of soluble carbohydrates within the seed coats. The marked reduction in oligosaccharide level and the disappearance of free hexoses from these tissues following imbibition indicate the possible participation of the seed coats in the carbohydrate metabolism of germination. Further, the sudden increase in sucrose levels in the cotyledons prior to the emergence of the apical meristem, and the marked decrease in levels of fructose and glucose in the hypocotyl following apical meristem growth illustrates that the hypocotyl functions both as an energy sink and an energy source during germination.

Studies on the time sequence of carbohydrate changes provided a better

understanding of the role of these compounds in germinating soybeans. This was illustrated by the finding that oligosaccharides decreased markedly during the first 12 hours of germination and increased to their original levels 6 hours thereafter. Such a finding provides a further insight into germination in that it implies the de novo syntheses of carbohydrates within the cotyledons and the concurrent mobilization and transfer of these metabolites to the embryonic axis. This situation was later demonstrated by the ^{14}C-experiments. Cotyledons were shown to synthesize sucrose, raffinose, and stachyose from exogenously administered ^{14}C-hexoses. Such findings had not hitherto been recognized.

The identification and quantitation of the soluble carbohydrates during germination provided an insight into the relative importance of these sugars in any one organ during germination. Perhaps the most useful finding here was that sucrose was the preferred form of carbohydrate storage in the cotyledons and that fructose and glucose were the predominant sugars of the growing parts. Also, the finding that fructose levels within the radicle were consistently greater than glucose levels might form the basis for further studies on the metabolism of germinating soybeans.

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